CENTERS FOR DISEASE CONTROL AND PREVENTION

DEPARTMENT OF HEALTH AND HUMAN SERVICES

UNITED STATES OF AMERICA

Ebola Information Packet for International Laboratories

RELEASE TO THE PUBLIC

VERSION 1.0

AUGUST 2014

August 20, 2014

Centers for Disease Control and Prevention
Atlanta, GA USA 30333

Dear Colleagues,

We have had a large number of requests for information on the Ebola virus outbreak in West Africa. This information packet provides:

- Information on Ebola virus disease (EVD)
- Information on CDC's EVD molecular diagnostic testing assays
- How to consult with CDC about EVD patients and decisions about testing
- How to submit diagnostic specimens directly to CDC for testing

Key points:

- If you suspect you may have a patient with Ebola virus disease, please follow your national and local guidance for notification and consultation for Ebola virus testing before contacting the CDC
- After this initial consultation, communication between the Ministry of Health, the WHO regional liaison, and the CDC can be initiated
- If the decision is made after the MoH, WHO, and CDC consultation to ship specimens to CDC for testing, CDC will supply further detailed guidance on Import permits, submission forms, and shipping and packing requirements
- Specimens received at CDC without prior consultation will not be tested. To ensure that testing is not delayed, please provide the parcel tracking information to: eocevent246@cdc.gov

Points of Contact for Consultation:

CDC Emergency Operations Center 24/7 hotline: +001-770-488-7100 email: **EOCEVENT240@cdc.gov**

Contents:

Section 1: Ebola Overview and Case Definitions

- Brief Introduction to Ebola virus and Laboratory Diagnostic assays
- CDC Ebola virus Case Definitions
- Summary of Web links for further information

Section 2: Laboratory Guidance Standard Operation Procedures and Protocols

- CDC Interim Guidance for Specimen collection, transport, testing and Submission for patients with Suspected Infection with Ebola virus
- **PROTOCOL:** VSPB-1100 Inactivation of enveloped RNA viruses and Extraction of Genomic material (RNA) using TriPure or Trizol Reagents and the Qiagen RNeasy kit
- **PROTOCOL:** VSPB-1101 Ebola virus (Zaire) nucleoprotein Real Time Reverse Transcription Polymerase Chain Reaction (Real Time RT-PCR)

Section 3: Decision Algorithms and Shipping Guidelines and Requirements to submit specimens to CDC for confirmatory testing

- Laboratory Testing Frequently Asked Questions (FAQ)
- CDC Ebola virus Testing Algorithm and Decision Tree
- CDC Diagnostic Specimen Shipping process overview and flow chart
- **Three (3) FORMS REQUIRED for TESTING BY CDC and SHIPPING to USA**
    - Public Health Service Importation Permit (will be provided after consultation)
    - CDC Viral Special Pathogens Specimen Submission Form
    - IATA Dangerous Goods Declaration Form
- IATA Category A Packaging Guide
    - Acceptance Checklist for Dry ice packaging

Section 4: Biosafety Guidance

- WHO Guidance document on Biosafety and Viral hemorrhagic fevers for International Laboratories and Hospitals

CENTERS FOR DISEASE CONTROL AND PREVENTION

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Ebola Overview & Case Definitions

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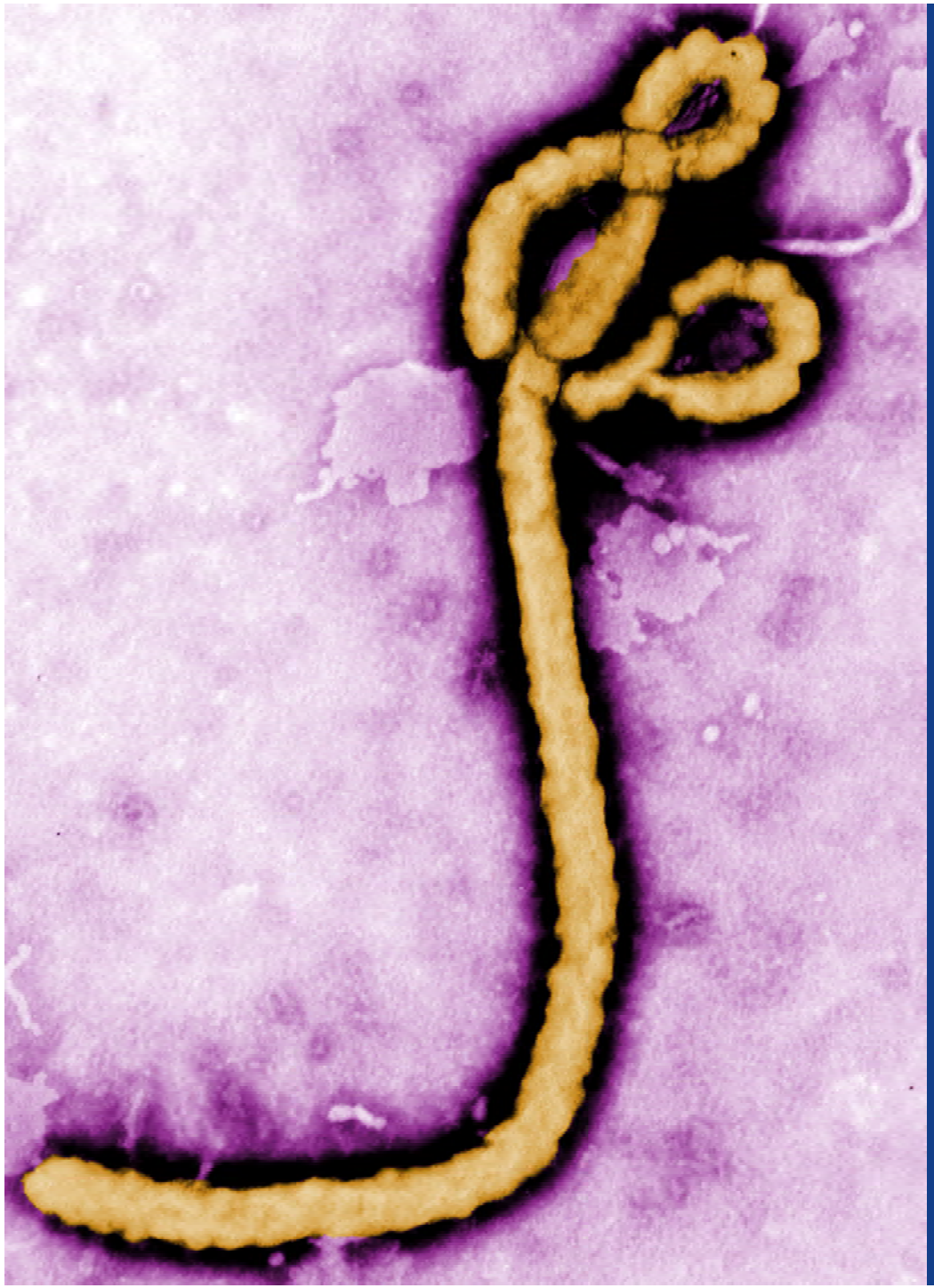


CDC 24/7

Ebola virus Overview

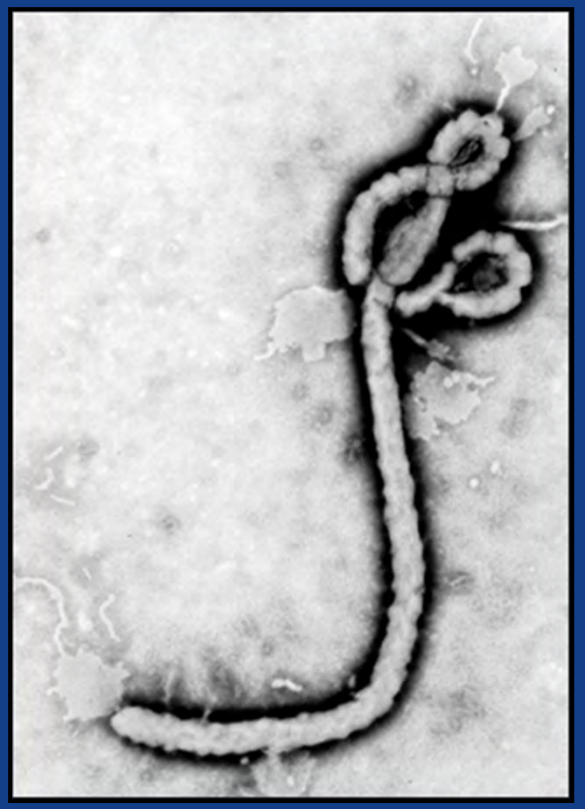
August 20th, 2014

Background: West Africa Ebola Outbreak

- **Largest ever recorded – Ebola (Zaire) virus**
  - >2,000 cases since March, >1000 deaths
  - First outbreak in West Africa: Guinea, Liberia, Sierra Leone, Nigeria
- **CDC Emergency Operations Center activated, staff and numerous international personnel deployed**
  - Clinical Care, Data Management, Epidemiology, Health Education & Communications, Laboratory diagnostics

Ebola and Marburg Hemorrhagic Fevers: The Viruses

- **Family *Filoviridae***
  - 5 species of *Ebolavirus*
    - *Zaire ebolavirus* (1976)= Ebola virus
    - *Sudan ebolavirus* (1976)= Sudan virus
    - *Reston ebolavirus* (1989)= Reston virus
    - *Taï Forest ebolavirus* (1994)= Tai Forest virus
    - *Bundibugyo ebolavirus* (2007)= Bundibugyo virus
  - 1 species of *Marburgvirus*
    - *Marburgvirus marburgvirus* (1967)



Ebolavirus Ecology

Enzootic Cycle

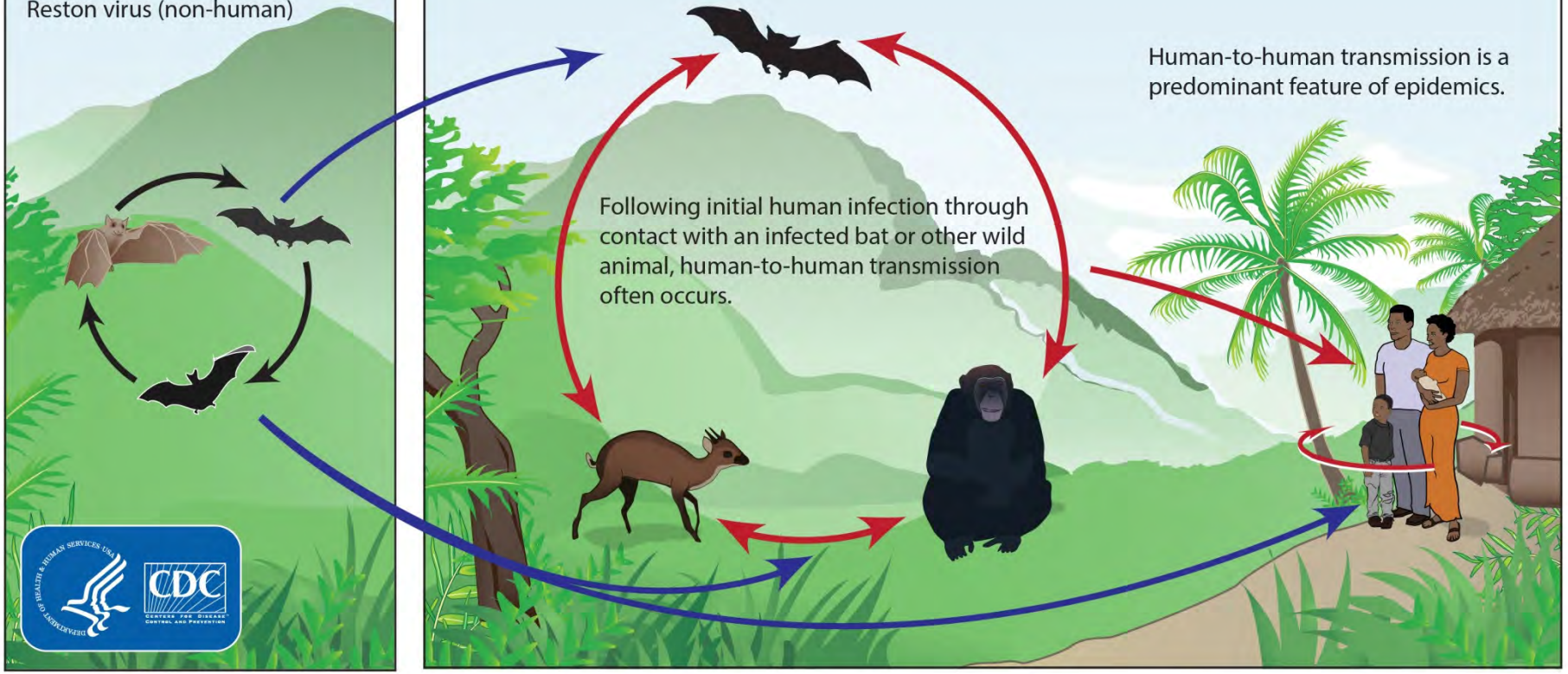
New evidence strongly implicates bats as the reservoir hosts for ebolaviruses, though the means of local enzootic maintainance and transmission of the virus within bat populations remain unknown.

Ebolaviruses:

Ebola virus (formerly Zaire virus)
Sudan virus
Taï Forest virus
Bundibugyo virus
Reston virus (non-human)

Epizootic Cycle

Epizootics caused by ebolaviruses appear sporadically, producing high mortality among non-human primates and duikers and may precede human outbreaks. Epidemics caused by ebolaviruses produce acute disease among humans, with the exception of Reston virus which does not produce detectable disease in humans. Little is known about how the virus first passes to humans, triggering waves of human-to-human transmission, and an epidemic.



Filovirus Ecology

- Marburg: reservoir is Egyptian Fruit Bats (*Rousettus aegyptiacus*)
  - Exposures to cave-dwelling bats
- Ebola: Reservoir is UNKNOWN
  - Bats implicated
- Non-Human primates also develop severe hemorrhagic disease
- Duikers, pigs (Reston)– can become infected, role of transmission unknown

Zoonotic Transmission

- Direct contact (slaughter, consumption) of infected animals:
  - Bats (are a possible source of infection)
  - Primates
- Environmental exposure
  - Entering caves, buildings infested with bats

Virus Inactivation

- 1:10 Bleach solution
- 5% Hospital-grade Lysol / phenolics
- Micro-Chem / quaternary ammonium
- Incineration / Autoclave
- Virus labile to desiccation and UV

Clinical Manifestations- Humans

- Incubation period: 2–21 days
- Abrupt onset
  - Fever, headache, chills, malaise, and myalgia
  - GI symptoms most common: vomiting, diarrhea, abdominal pain
  - Hemorrhagic symptoms in <half of cases
    - Mild: petechiae, epistaxis, ecchymosis, bruising
    - Severe: GI hemorrhage, shock, DIC
  - Less commonly seen: rash (trunk, shoulders), conjunctivitis, pharyngitis, cough, hiccups

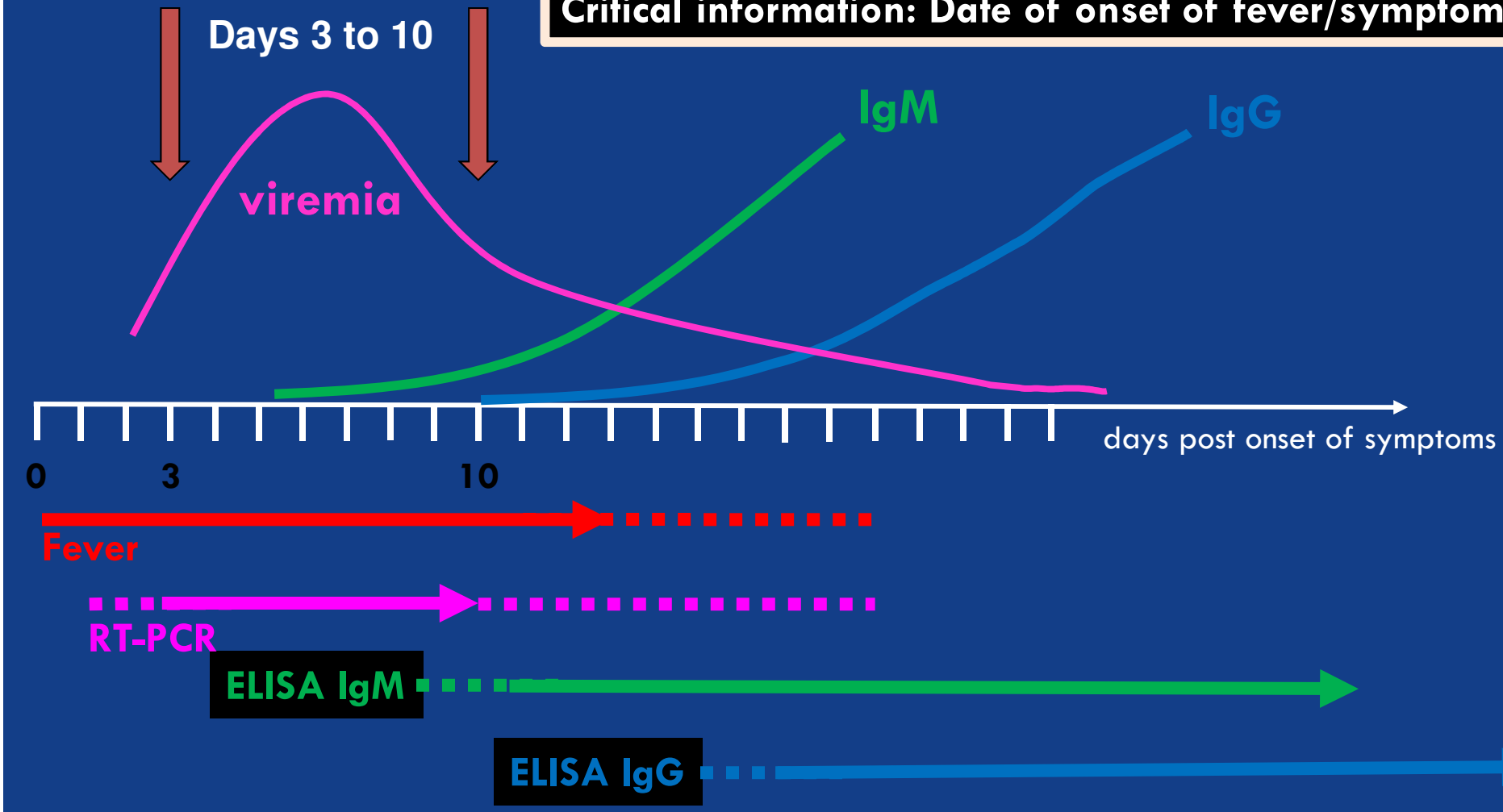
Course of Disease & Virus shedding

- Not transmissible prior to onset of symptoms
  - All body fluids (blood, urine, saliva, feces etc.) can carry virus
- Virus quantity increases to death, usually 9-10 days post-onset of symptoms
- If patient survives to day 14, increased chance of survival
- Convalescence/resolution of viremia

LABORATORY DIAGNOSTICS

Laboratory Diagnosis

Critical information: Date of onset of fever/symptoms



IgM: up to 3 – 6 months

IgG: up to 3 – 5 years (life-long persistance?)

General Laboratory Diagnostics

- Appropriate test depends on the timing of the sample (diagnostic testing is useless unless there are symptoms present)
- Blood and sera are best specimens for testing in live patients
- Tissues (spleen, liver, skin snips) may be tested if patient is deceased.
- Oral swabs can also be used in extreme circumstances but is not recommended for routine testing (not as sensitive, shorter window for positives, etc)

General Laboratory Diagnostics

- Please note, diagnostic testing is useless unless there are symptoms present (Testing in the absence of symptoms is dangerous)
- General diagnostic tests (some require special facilities):
  - Molecular detection – RT-PCR and Real-time RT-PCR (detects virus)
    - Ideally on specimens from days 3 to 10 post-onset of symptoms
  - Viral isolation* – only in BSL4 laboratory
  - Antigen ELISA (detects virus)
  - IgM ELISA (detects early antibody)
  - IgG ELISA (detects late antibody)

Diagnostic Shipping Guidelines

- PLEASE CONTACT THE CDC EOC
- +1-770-488-7100
- Eocevent246@cdc.gov

- 4 mL serum, plasma, or whole blood
- Shipped refrigerated on ice pack or frozen on dry ice (no glass tubes)
- IATA guidelines; triple-packaging
- UN 2814 “Suspected Category A Infectious Substance”

Centers for Disease Control and Prevention

Case Definition for Ebola Virus Disease (EVD)

Updated: August 22, 2014

Early recognition is critical for infection control. Health care providers should be alert for and evaluate any patients suspected of having Ebola Virus Disease (EVD).

Person Under Investigation (PUI)

A person who has both consistent symptoms and risk factors as follows:

1. Clinical criteria, which includes fever of greater than 38.6 degrees Celsius or 101.5 degrees Fahrenheit, and additional symptoms such as severe headache, muscle pain, vomiting, diarrhea, abdominal pain, or unexplained hemorrhage; AND
2. epidemiologic risk factors within the past 21 days before the onset of symptoms, such as contact with blood or other body fluids or human remains of a patient known to have or suspected to have EVD; residence in—or travel to—an area where EVD transmission is active*; or direct handling of bats or non-human primates from disease-endemic areas.

Probable Case

A PUI whose epidemiologic risk factors include high or low risk exposure(s) (see below)

Confirmed Case

A case with laboratory-confirmed diagnostic evidence of Ebola virus infection

Exposure Risk Levels

Levels of exposure risk are defined as follows:

High risk exposures

A high risk exposure includes any of the following:

- Percutaneous (e.g., needle stick) or mucous membrane exposure to blood or body fluids of EVD patient

- Direct skin contact with, or exposure to blood or body fluids of, an EVD patient without appropriate personal protective equipment (PPE)
- Processing blood or body fluids of a confirmed EVD patient without appropriate PPE or standard biosafety precautions
- Direct contact with a dead body without appropriate PPE in a country where an EVD outbreak is occurring*

Low[1] risk exposures

A low risk exposure includes any of the following

- Household contact with an EVD patient
- Other close contact with EVD patients in health care facilities or community settings. Close contact is defined as
  a. being within approximately 3 feet (1 meter) of an EVD patient or within the patient's room or care area for a prolonged period of time (e.g., health care personnel, household members) while not wearing recommended personal protective equipment (i.e., standard, droplet, and contact precautions; see Infection Prevention and Control Recommendations (/vhf/ebola/hcp/patient-management-us-hospitals.html))
  b. having direct brief contact (e.g., shaking hands) with an EVD case while not wearing recommended personal protective equipment.
- Brief interactions, such as walking by a person or moving through a hospital, do not constitute close contact

No known exposure

Having been in a country in which an EVD outbreak occurred within the past 21 days and having had no high or low risk exposures

* As of 22 August 2014, countries with EVD outbreaks are Guinea, Liberia, and Sierra Leone. There are also cases of EVD in Lagos, Nigeria. For more information about specific districts where the EVD outbreak is occurring, visit: www.cdc.gov/vhf/ebola/outbreaks/guinea/ (/vhf/ebola/outbreaks/guinea/index.html)

[1] For purposes of monitoring and movement restrictions of persons with Ebola virus exposure, low risk is interpreted as some risk. See www.cdc.gov/vhf/ebola/hcp/monitoring-and-movement-of-persons-with-exposure.html (/vhf/ebola/hcp/monitoring-and-movement-of-persons-with-exposure.html)

Page last reviewed: August 22, 2014
Page last updated: August 22, 2014

Content source: Centers for Disease Control and Prevention (/index.htm)
National Center for Emerging and Zoonotic Infectious Diseases (NCEZID) (/ncezid/index.html)
Division of High-Consequence Pathogens and Pathology (DHCPP) (/ncezid/dhcpp/index.html)
Viral Special Pathogens Branch (VSPB) (/ncezid/dhcpp/vspb/index.html)

Web-links for Information on Ebola Hemorrhagic fever

[Contact CDC EOC for official guidance on Ebola: Eocevent240@cdc.gov]

Fact sheets:

CDC Fact sheet: http://www.cdc.gov/vhf/ebola/index.html

WHO fact sheet: http://who.int/mediacentre/factsheets/fs103/en/

Case definitions:

Case definition CDC: http://www.cdc.gov/vhf/ebola/hcp/case-definition.html

Case definition WHO: http://who.int/csr/resources/publications/ebola/ebola-case-definition-contact-en.pdf?ua=1

Infection control:

CDC: http://www.cdc.gov/vhf/ebola/hcp/infection-prevention-and-control-recommendations.html

WHO Risk assessment: http://who.int/csr/disease/ebola/EVD_WestAfrica_WHO_RiskAssessment_20140624.pdf?ua=1

Public awareness documents

http://www.cdc.gov/vhf/ebola/outbreaks/guinea/print-resources.html

HAN:

Health Alert Network (HAN): Recent HAN; HAN 364; HAN 365; HAN 366; HAN 367

Laboratory diagnosis:

Diagnostic information CDC: http://www.cdc.gov/vhf/ebola/diagnosis/index.html

CDC Sample collection guidelines: http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html

Form for specimen submission with in the US: http://www.cdc.gov/ncezid/dhcpp/vspb/specimens.html

http://www.cdc.gov/ncezid/dhcpp/vspb/pdf/specimen-submission.pdf

Reference Laboratories:

1) **CDC, Atlanta GA, USA**
2) **Pasteur Institute, Lyon, France**
3) **BNI Hamburg, Germany**
4) **National Microbiology Laboratory, Winnipeg, Canada**

CENTERS FOR DISEASE CONTROL AND PREVENTION

DEPARTMENT OF HEALTH AND HUMAN SERVICES

UNITED STATES OF AMERICA

Laboratory Standard Operating Procedures and Protocols

RELEASE TO THE PUBLIC

VERSION 1.0

AUGUST 2014

Centers for Disease Control and Prevention

Interim Guidance for Specimen Collection, Transport, Testing, and Submission for Patients with Suspected Infection with Ebola Virus Disease

On this Page

Background

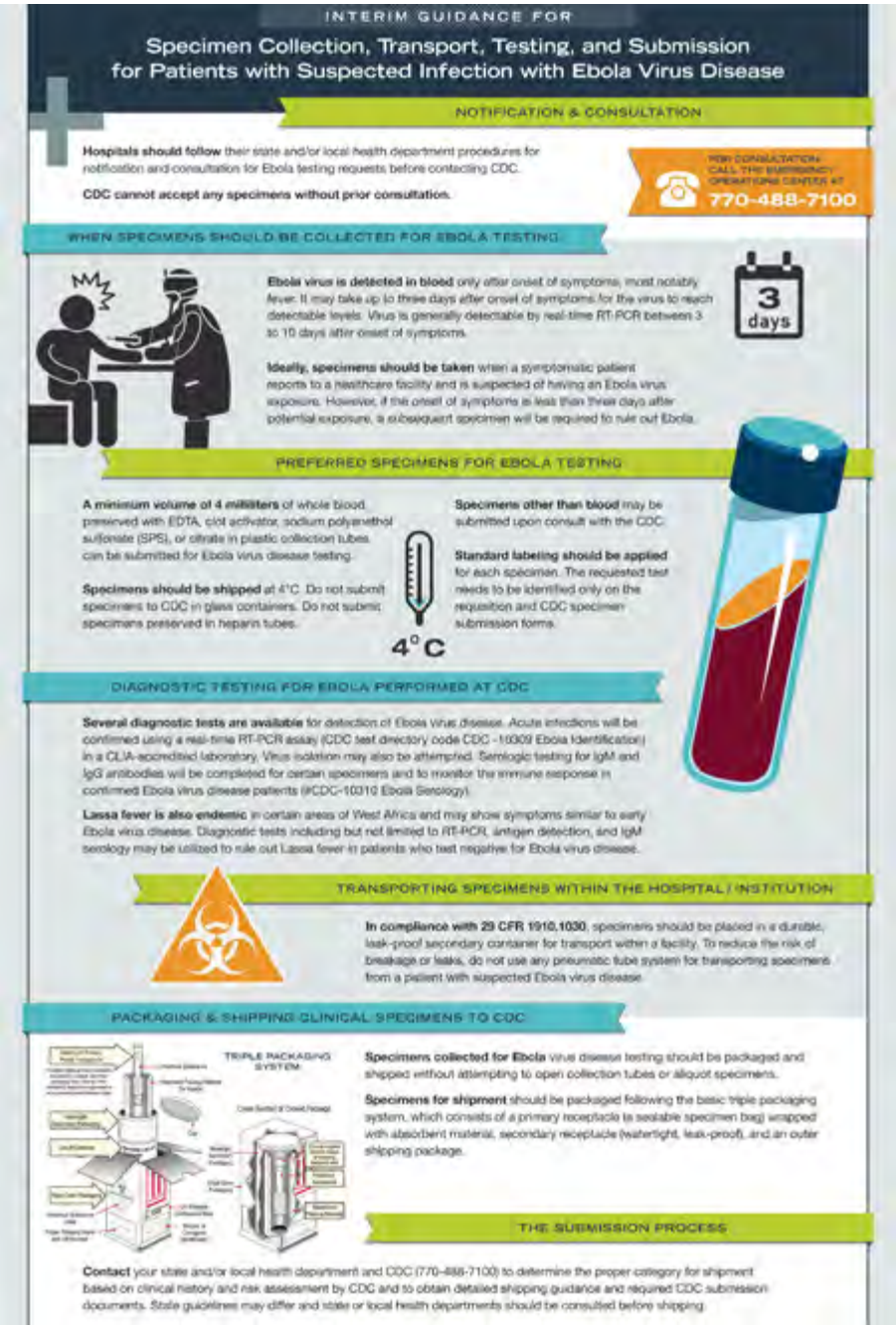
CDC is working with the World Health Organization (WHO), the ministries of health, and other international organizations in response to an outbreak of EVD in West Africa, which was first reported in late March 2014. This is the largest outbreak of Ebola virus disease (EVD) ever documented and the first recorded in West Africa. For the latest information on the outbreak, see the 2014 Ebola Outbreak in West Africa (/vhf/ebola/outbreaks/guinea/index.html) highlights.

EVD is one of numerous viral hemorrhagic fevers (VHF). It is a severe, often fatal disease in human and nonhuman primates. EVD is spread by direct contact with the blood or secretions (urine, feces, semen, breast milk, and possibly others) of an infected person or exposure to objects that have been contaminated with infected secretions. The incubation period is usually 8 –10 days (rarely ranging from 2 to 21 days). Patients can transmit the virus while febrile and through later stages of disease, as well as postmortem.

U.S. hospitals can safely manage a patient with EVD by following recommended isolation and infection control procedures (/vhf/ebola/hcp/infection-prevention-and-control-recommendations.html). Standard, contact, and droplet precautions are recommended for management of hospitalized patients with known or suspected (/vhf/ebola/hcp/case-definition.html) EVD.

Potentially infectious diagnostic specimens are routinely handled and tested in U.S. laboratories in a safe manner, through adherence to standard safety precautions as outlined below.

Purpose



(/vhf/ebola/pdf/ebola-lab-guidance.pdf)

Printable factsheet: Interim Guidance for Specimen Collection, Transport, Testing, and Submission for Patients with Suspected Infection with Ebola Virus Disease [PDF - 1 page]

(/vhf/ebola/pdf/ebola-lab-guidance.pdf)

This document provides interim guidance for laboratorians and other healthcare personnel collecting or handling specimens in the United States on appropriate specimen collection, transport and testing of specimens from patients who are suspected to be infected with Ebola virus.

Infection Control for Collecting and Handling Specimens

It is expected that all laboratorians and other healthcare personnel collecting or handling specimens follow established standards compliant with the OSHA bloodborne pathogens standard (https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=10051&p_table=STANDARDS), which encompasses blood and other potentially infectious materials. This includes wearing appropriate personal protective equipment (PPE) and adhering to engineered safeguards, for all specimens regardless of whether they are identified as being infectious.

Recommendations for specimen collection: full face shield or goggles, masks to cover all of nose and mouth, gloves, fluid resistant or impermeable gowns. Additional PPE may be required in certain situations.

Recommendations for laboratory testing: full face shield or goggles, masks to cover all of nose and mouth, gloves, fluid resistant or impermeable gowns AND use of a certified class II Biosafety cabinet or plexiglass splash guard, as well as manufacturer-installed safety features for instruments.

Specimen Handling for Routine Laboratory Testing (not for Ebola Diagnosis)

Routine laboratory testing includes traditional chemistry, hematology, and other laboratory testing used to support and treat patients. Precautions as described above offer appropriate protection for healthcare personnel performing laboratory testing on specimens from patients with suspected infection with Ebola virus. These precautions include both manufacturer installed safety features for instruments and the environment as well as PPE specified in the box above.

When used according to the manufacturer's instructions, Environmental Protection Agency (EPA)-registered disinfectants routinely used to decontaminate the laboratory environment (benchtops and surfaces) and the laboratory instrumentation are sufficient to inactivate enveloped viruses, such as influenza, hepatitis C, and Ebola viruses.

When Specimens Should Be Collected for Ebola Testing

Ebola virus is detected in blood only after onset of symptoms, most notably fever. It may take up to 3 days post-onset of symptoms for the virus to reach detectable levels. Virus is generally detectable by real-time RT-PCR from 3-10 days post-onset of symptoms, but has been detected for several months in certain secretions. Specimens ideally should be taken when a symptomatic

patient reports to a healthcare facility and is suspected of having an EVD exposure; however, if the onset of symptoms is <3 days, a subsequent specimen will be required to completely rule-out EVD.

Preferred Specimens for Ebola Testing

A minimum volume of 4mL whole blood preserved with EDTA, clot activator, sodium polyanethol sulfonate (SPS), or citrate in ***plastic*** collection tubes can be submitted for EVD testing. Do not submit specimens to CDC in glass containers. Do not submit specimens preserved in heparin tubes. Specimens should be stored at 4°C or frozen. Specimens other than blood may be submitted upon consult with the CDC by calling the Emergency Operations Center at 770-488-7100.

Standard labeling should be applied for each specimen. The requested test only needs to be identified on the requisition and CDC specimen submission forms.

Storing Clinical Specimens for Ebola

Specimens should be stored at 4°C or frozen.

Diagnostic Testing for Ebola Performed at CDC

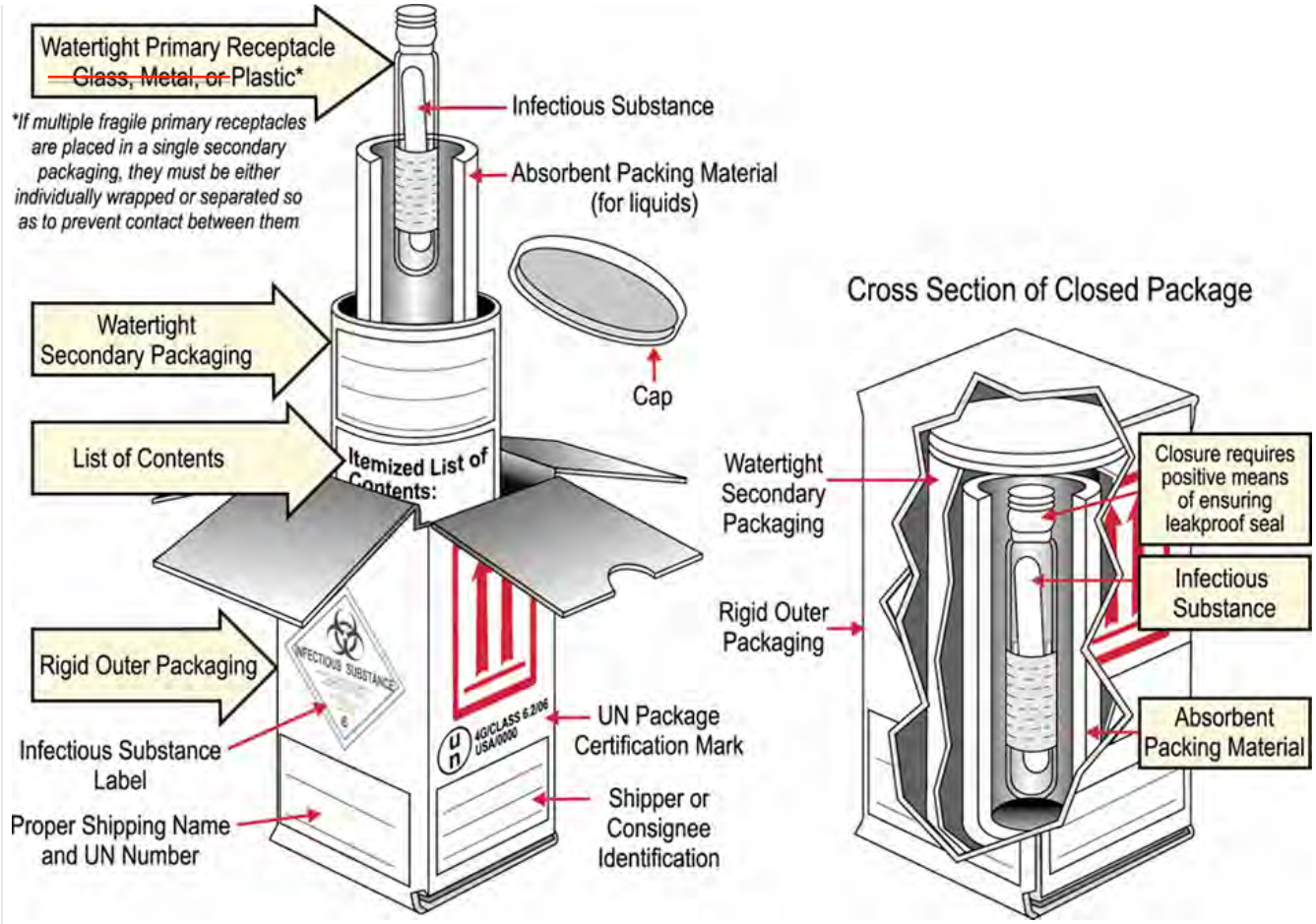
Several diagnostic tests are available for detection of EVD. Acute infections will be confirmed using a real-time RT-PCR assay (CDC test directory code CDC -10309 Ebola Identification) in a CLIA-accredited laboratory. Virus isolation may also be attempted. Serologic testing for IgM and IgG antibodies will be completed for certain specimens and to monitor the immune response in confirmed EVD patients (#CDC-10310 Ebola Serology).

Lassa fever is also endemic in certain areas of West Africa and may show symptoms similar to early EVD. Diagnostic tests including but not limited to RT-PCR, antigen detection, and IgM serology may be utilized to rule out Lassa fever in EVD-negative patients.

Transporting Specimens within the Hospital / Institution

In compliance with 29 CFR 1910.1030, specimens should be placed in a durable, leak-proof secondary container for transport within a facility. To reduce the risk of breakage or leaks, do not use any pneumatic tube system for transporting suspected EVD specimens.

Packaging and Shipping Clinical Specimens to CDC



(/vhf/ebola/hcp/packaging-diagram.html)

PACKAGING DIAGRAM (/VHF/EBOLA/HCP/PACKAGING-DIAGRAM.HTML)

Specimens collected for EVD testing should be packaged and shipped without attempting to open collection tubes or aliquot specimens. Specimens for shipment should be packaged following the basic triple packaging system which consists of a primary receptacle (a sealable specimen bag) wrapped with absorbent material, secondary receptacle (watertight, leak-proof), and an outer shipping package. See diagram » (/vhf/ebola/hcp/packaging-diagram.html)

The following steps outline the submission process to CDC.

- Hospitals should follow their state and/or local health department procedures for notification and consultation for Ebola testing requests and prior to contacting CDC.
- NO specimens will be accepted without prior consultation. For consultation call the EOC at **770-488-7100**.
- Contact your state and/or local health department and CDC to determine the proper category for shipment based on clinical history and risk assessment by CDC. State guidelines may differ and state or local health departments should be consulted prior to shipping.

- Email tracking number to **EOCEVENT246@CDC.GOV (mailto:EOCEVENT246@CDC.GOV)**.
- Do not ship for weekend delivery unless instructed by CDC.
- Ship to:

Centers for Disease Control and Prevention
ATTN STAT LAB: VSPB, UNIT #70
1600 Clifton Road NE
Atlanta, GA 30333
Phone 770-488-7100

- Include the following information: your name, the patient's name, test(s) requested, date of collection, laboratory or accession number, and the type of specimen being shipped.
- Include the CDC Infectious Disease (CDC Form 50.34 (http://www.cdc.gov/laboratory/specimen-submission/form.html)) and Viral Special Pathogens Branch [PDF - 2 pages] (/ncezid/dhcpp/vspb/pdf/specimen-submission.pdf) specimen submission forms.
- On the **outside** of the box, specify how the specimen should be stored: **refrigerated** or **frozen**.

Occupational Health

Potential exposures to blood, body fluids and other infectious materials must be reported immediately according to your institution's policy and procedures.

When to Contact CDC

CDC highly recommends contacting your state and/or local health department prior to contacting CDC.

CDC is available for consultation 24/7 at 770-488-7100.

CDC will continue to evaluate new information as it becomes available and will update this guidance as needed.

Additional Resources and Information

- http://www.cdc.gov/ncezid/dhcpp/vspb/pdf/specimen-submission.pdf [PDF - 2 pages] (/ncezid/dhcpp/vspb/pdf/specimen-submission.pdf)
- http://www.cdc.gov/ncezid/dhcpp/vspb/specimens.html (/ncezid/dhcpp/vspb/specimens.html)

- http://www.cdc.gov/vhf/ebola/hcp/infection-prevention-and-control-recommendations.html (/vhf/ebola/hcp/infection-prevention-and-control-recommendations.html)
- http://content.govdelivery.com/accounts/USCDC/bulletins/c7bea0 (http://content.govdelivery.com/accounts/USCDC/bulletins/c7bea0)
- http://www.cdc.gov/hicpac/disinfection_sterilization/6_0disinfection.html (http://www.cdc.gov/hicpac/disinfection_sterilization/6_0disinfection.html)
- http://www.cdc.gov/mmwr/pdf/other/su6101.pdf [PDF - 105 pages] (http://www.cdc.gov/mmwr/pdf/other/su6101.pdf)
- http://www.cdc.gov/laboratory/specimen-submission/form.html (http://www.cdc.gov/laboratory/specimen-submission/form.html)

File Formats Help:

How do I view different file formats (PDF, DOC, PPT, MPEG) on this site? (http://www.cdc.gov/Other/plugins/)

(http://www.cdc.gov/Other/plugins/#pdf)

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National Center for Emerging and Zoonotic Infectious Diseases (NCEZID) (/ncezid/index.html)
Division of High-Consequence Pathogens and Pathology (DHCPP) (/ncezid/dhcpp/index.html)
Viral Special Pathogens Branch (VSPB) (/ncezid/dhcpp/vspb/index.html)

Viral Special Pathogens Branch
Centers for Disease Control and Prevention

LABORATORY DIAGNOSTIC STANDARD OPERATING PROCEDURE

Facility:	
Title: Inactivation of Enveloped RNA Viruses and Extraction of Genomic Material (RNA) using Tripure or Trizol Reagents and the Qiagen RNeasy Kit	
Document Number: VSPB-1100	**Effective Date:** 08/18/2014 **Version Number:** 1

Other documents cross-referenced in this SOP:

SOP# VSPB1101: Ebola virus (Zaire) nucleoprotein Real Time Reverse Transcription Polymerase Chain Reaction (Real Time RT-PCR)

REVISION HISTORY

Rev #	DCR #	Changes Made to Document	Date
new document	VSPB-1100	Protocol adapted from SOP# VSPB-VI -1004 and SOP# MD-1001, to provide to International Partner Organizations and Laboratories; SA, BB, MR	08/18/2014
Reviewed by:			

I. PURPOSE

This protocol describes the procedure for inactivation of filoviruses, arenaviruses, bunyaviruses, and paramyxoviruses; and the subsequent RNA extraction procedure. The inactivation procedure removes infectivity to ensure the safety of laboratory personnel handling the samples, while preserving virus genome. The RNA Extraction procedure isolates purified RNA from the inactivated biological matrices (samples) for subsequent genomic analyses such as RT-PCR diagnostics and genome sequencing.

Samples appropriate for testing include whole blood, sera, and plasma from humans or animals.

This procedure assumes a basic familiarity with nucleic acid handling.

II. PRINCIPLE

For RNA extraction, the sample is first lysed under highly denaturing conditions to remove infectivity and inactivate RNases, while ensuring isolation of intact viral RNA. Sample inactivation is accomplished through use of a one-step sample homogenization/lysis procedure, in which the TriPure Isolation Reagent disrupts cells and denatures endogenous nucleases. After chloroform is added to the extract, the mixture is centrifuged and separates into three phases: a colorless aqueous (upper) phase, a white interphase and a red organic (lower) phase. The phases may then be separated and RNA recovered from the colorless aqueous phase, and DNA or protein from the interphase and red organic phase.

Note: Published studies and in-house CDC/VSPB testing have documented the inactivation of enveloped RNA viruses by Tripure.

Buffering conditions are then adjusted to provide optimum binding of the RNA to the Qiagen spin column. The RNA binds to the column membrane, and contaminants are efficiently washed away in two steps using two different wash buffers. High-quality RNA is eluted in a special RNase-free buffer, ready for direct use or safe storage. The purified RNA is free of protein, nucleases, and other contaminants and inhibitors.

III. RESPONSIBILITIES

A. Staff Responsibilities

Position	Responsibility
Biosafety Officer	-Reviews this SOP and performs a risk assessment for the work areas intended to conduct it; -Reviews and approves designated locations for sample preparation, storage and testing activities for the site

	where each is to take place; Ensures staff is adequately trained in biosafety procedures.
Quality Officer	-Understands and enforces relevant certification information (agency or country-specific) as applicable to execution of this SOP. -Monitors adherence to this SOP by the personnel conducting it and oversees occurrence management where necessary.
Laboratory Manager	- Ensures appropriate inactivation and extraction protocols have been used and documented; - Ensures retention of applicable logs and worksheets; - Ensures testing personnel have the appropriate training.
Testing Personnel	- Adheres to all SOP instructions and biosafety requirements; - Documents completion of sample inactivation and other procedural steps, as applicable.

B. Specific Safety and Containment Requirements and Responsibilities

This section specifies actions and considerations to be taken into account according to the risk assessment conducted by the Biosafety Officer, such as known hazards specific to this SOP, and biosafety practices required for the safe execution of its procedures and use of reagents. **Adherence to appropriate safety guidelines and related biosafety SOPs is mandatory.**

WARNING: YOU MUST INACTIVATE VIRUS USING THE RECOMMENDED TRIPURE OR TRIZOL REAGENTS.

DO NOT SKIP THIS CRITICAL INACTIVATION STEP.

Note-- the following guidelines are excerpted from the World Health Organization's publication: *Interim Infection Prevention and Control Guidance for Care of Patients with Suspected or Confirmed Filovirus Haemorrhagic Fever in Health-Care Settings, with Focus on Ebola*, and from the *WHO Biosafety Manual*.

- Laboratory personnel should wear appropriate personal protective equipment (PPE), to include:
  - Closed shoes (no sandals) with disposable shoe covers, or laboratory boots.
  - Disposable gloves. Consider double gloving when the quality of gloves appears to be poor (e.g., if holes and tears form rapidly during use.
  - A disposable, impermeable gown with full-length sleeves. Front-buttoned standard laboratory coats are unsuitable, as are sleeves that do not fully

cover the forearms; use of disposable sleeves with standard lab coats do not proved a sufficient barrier.
    - Medical mask and eye protection (eye visor, or goggles, or face shield).
    - When aliquoting or undertaking any other procedure that may generate aerosols in the absence of other physical barriers such as a biosafety cabinet, particulate respirators (e.g., FFP2, or EN certified equivalent, or US NIOSH-certified N95), or powered air purifying respirators (PAPR) should be used.
- Laboratory protective clothing must not be worn outside the laboratory, and it must be decontaminated before disposal. The removal of street clothing and change into dedicated laboratory clothing in addition to the use of disposable outer wear may be warranted based on institutional practice.
- Dispose of pipette tips and other sharp objects in appropriate, puncture-resistant containers with disinfectant.
    - Ensure that puncture-resistant containers for sharps objects are placed as close as possible to the immediate area where the objects are being used ('point of use') to limit the distance between use and disposal, and ensure the containers remain upright at all times. Ensure that the puncture-resistant containers are securely sealed with a lid and replaced when 3/4 full.
- When removing PPE, avoid any contact between the soiled items (e.g. gloves, gowns) and any area of the face (i.e. eyes, nose or mouth).
- Do not hang up the apron or gown for reuse- discard immediately.
- Perform hand hygiene immediately after the removal of PPE used during specimen handling and after any contact with potentially contaminated surfaces even when PPE is worn.
- Place specimens in clearly-labelled, non-glass, leak-proof containers and deliver directly to designated specimen handling areas.
- Disinfect all external surfaces of specimen containers thoroughly (using an effective disinfectant) prior to transport. The Ebola virus can be eliminated with denaturing detergents and sodium hypochlorite (bleach) or calcium hypochlorite (bleaching powder) at appropriate concentrations. (See Attachment D for further instructions).
- For Waste Management:
    - Wear heavy duty/rubber gloves, impermeable gown, closed shoes (e.g. boots) and facial protection (mask and goggle or face shield), when handling infectious waste (e.g. solid waste or any secretion or excretion with visible blood, even if it originated from a normally sterile body cavity).
    - Goggles with medical mask provide greater protection than visors with medical mask, from splashes that may come from below when pouring liquid waste from a bucket. Avoid splashing when disposing of liquid infectious waste.
    - Waste should be segregated at point of generation to enable appropriate and safe handling.

- Sharp objects (e.g. needles, syringes, glass articles) and tubing that has been in contact with blood or body fluids should be placed inside puncture resistant waste containers. These should be located as close as practical to the area where the items are used.
- Collect all solid, non-sharp, infectious waste using leak-proof waste bags and covered bins. Bins should never be carried against the body (e.g. on the shoulder).
- A fire pit or incinerator may be used for short periods during an outbreak to destroy solid waste. However, it is essential to ensure that total incineration has taken place. Caution is also required when handling flammable material and when wearing gloves due to the risk of burn injuries if gloves are ignited.
- Autoclaved or incinerated waste should be placed in a designated pit of appropriate depth (e.g. 2 m or about 7 feet) and filled to a depth of 1–1.5 m (or about 3–5 feet). After each waste load, the waste should be covered with a layer of soil 10 –15 cm deep.
- The area designated for the final treatment and disposal of waste should have controlled access to prevent entry by animals, untrained personnel or children.

- In case of exposure:
  - Persons with percutaneous or muco-cutaneous exposure to blood, body fluids, secretions, or excretions from a patient with suspected or confirmed Hemorrhagic Fever (HF) should immediately and safely stop any current tasks, and safely remove PPE. Remove PPE carefully because exposure during PPE removal can be just as dangerous for nosocomial transmission of HF.
  - Immediately wash the affected skin surfaces or the percutaneous injury site with soap and water. Accordingly, irrigate mucous membranes (e.g. conjunctiva) with copious amounts of water or an eyewash solution.
  - Immediately report the incident to Supervisor. Exposed persons should be medically evaluated, including for other potential exposures (e.g., HIV, HCV) and receive follow-up care, including fever monitoring, twice daily for 21 days after the incident.
  - Immediate consultation with an expert in infectious diseases is recommended for any exposed person who develops fever within 21 days of exposure.

Additional Precautions:

- Some of the reagents used to purify RNA are caustic. Read the MSDS sheets and be familiar with treatment protocols in case of accidental exposure and how to clean up any spills.
- TriPure Isolation Reagent contains phenol, which is highly caustic to skin.
- Do not mix TriPure Isolation Reagent with bleach. TriPure Isolation Reagent contains guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach.

- If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water.
- If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. Dispose of cleaning materials as biological waste.

IV. SAMPLE HANDLING

Samples appropriate for testing include whole blood, sera, and plasma from humans or animals.

Samples must be thoroughly evaluated to determine the proper initial processing area and handling procedures to be used. This should take into account the patient's clinical history, clinical signs, exposure risks, geographic area of origin, and any accompanying communications from the submitting physician or diagnostic laboratory.

For additional sample handling biosafety guidance, refer to:

World Health Organization. Interim Infection Prevention and Control Guidance for Care of Patients with Suspected or Confirmed Filovirus Haemorrhagic Fever in Health-Care Settings, with Focus on Ebola; Geneva: World Health Organization (WHO); 2014. Available from URL: http://www.who.int/csr/resources/who-ipc-guidance-ebolafinal-09082014.pdf?ua=1)

V. REAGENTS - MATERIALS/SUPPLIES – EQUIPMENT

A. Materials/Supplies/Equipment for Biosafety

BIOSAFETY
Reagents
• Standard hospital detergent • Disinfectant (minimum of 0.5% sodium hypochlorite – see Attachment D)* **Note:** 70% ethanol or methanol are not appropriate as disinfectants
Supplies
• Disposable single-use gloves • Disposable, impermeable gown • Disposable shoe covers or laboratory boots • Disposable mask • Eye protection or face shields to protect eyes, nose, mouth • Additional PPE as needed, based on risk assessment • Biohazard waste bags/container • Sharps container • Disinfectant container • Absorbent towels

Equipment
• Plexiglass shield or splashguards, at minimum • Class II Biosafety Cabinet, if available

***Note-- Chlorine solutions gradually lose strength, thus fresh solutions must be prepared daily.**

See Appendix D for instructions on how to prepare disinfectant (1 part 3.5% sodium hypochlorite + 6 parts water = 0.5% sodium hypochlorite solution)

B. Materials/Supplies/Equipment for Procedure

Molecular biology-grade reagents should be used and plastics should be sterile and certified RNAse/DNAse free.
Observe storage conditions and expiration dating as defined by the manufacturer.

PROCEDURE
Reagents
• Tripure Reagent (Roche Catalog # 11667157001), or Trizol (Invitrogen Catalog # 15596) • Qiagen RNeasy kit (Catalog # 74104), • Molecular grade sterile distilled water (RNAse and DNAse free) • Chloroform (isoamyl alcohol free – i.e., Sigma catalog #C2432 or equivalent
Supplies
• Screw cap microcentrifuge tubes • Pipette tips • Laboratory marking pen • Ice bucket containing ice for reaction or isolation tubes • 20µl, 200µl, and 1000µl adjustable pipettes and aerosol barrier tips • Sterile, nuclease-free 1.5ml microcentrifuge tubes
Equipment
• Vortex • Microcentrifuge with sealed rotorhead • Micropipettor

C. Reagent Preparation

1. Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution
2. Buffer RLT may form a precipitate upon storage. If necessary, re-dissolve by warming, and then place at room temperature (20–25°C).

IV. QUALITY ASSURANCE/QUALITY CONTROL

Quality Assurance

An established quality assurance (QA) program should be in place in order to minimize the risk of errors and to maximize the possibility of discovering errors if they occur. The institutional QA program must follow the recommendations from established standards organizations for quality assurance.

Prior to instituting this protocol, dedicated laboratory space which is physically segregated should be designated for each of the following activities and a risk assessment done of the work environment and processes. These include designated space for:

- Specimen receiving, accession, and storage;
- Sample inactivation and nucleic acid extraction;
- Master mix preparation (clean space);
- Reaction mixture and sample addition;
- Amplification and detection.

To avoid sample contamination:

- Always wear powder-free gloves when handling reagents.
- Never return an aliquot to the original container.
- Maintain separate areas for assay setup.
- Maintain separate areas for handling of nucleic acids.
- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., screw cap microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples whenever you suspect they may be contaminated.
- Keep reagent and reaction tubes capped or covered as much as possible.

General Equipment preparation:

- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach or “RNase Zap™” or equivalent, to minimize risk of nucleic acid contamination.

At each step, verify that the integrity of all sample information is maintained. If clerical errors are discovered, correct and document by initialing. If the integrity of sample identity is compromised at any step, processing must be repeated for the affected steps of the analysis. Document the actions taken. Specimen identity must be documented and verified at every step as part of standard operating procedure.

Record the lot/batch number of reagents and their expiration dates in applicable worksheets and logs.

Quality Control

Generally, total RNA will be isolated from samples. However, in any case where RNA selection occurs (e.g. isolation of cytoplasmic RNA only), total RNA must be isolated concurrently from negative control samples for use as template in subsequent control reactions (i.e. ß2-microglobulin control PCR).

It is recommended to run an internal extraction control such as RNase P or Beta 2 microglobulin (B2M), for every patient sample tested. Additionally, one Negative Extraction Control and one Negative PCR Control should be conducted in each run.

If controls do not perform as expected during PCR assay, the entire assay should be repeated, including extraction phase. A discordant control result may indicate a procedural error. Do not report test results until all problems have been resolved.

V. PROCEDURE

A. Procedure for Inactivation

WARNING: YOU MUST INACTIVATE VIRUS USING THE RECOMMENDED TRIPURE OR TRIZOL REAGENTS.

DO NOT SKIP THIS CRITICAL INACTIVATION STEP.

Note —before performing the following steps, a facility-based risk assessment must first be conducted, then the work done in accordance with WHO guidance.

All laboratory sample processing must take place under a Class II biosafety cabinet, or higher. **Do not carry out procedure on the open bench.**

Preparation of Equipment and Laboratory Work Area

1. Use appropriate personal protective equipment (PPE), according to risk assessment and as recommended in the Safety section of this SOP.

2. Prepare disinfectant (minimum of 0.5% sodium hypochlorite or other laboratory grade denaturing disinfectant) and add 100 ml of disinfectant to the disinfectant container.

3. Clean the work area where the tests will be performed with disinfectant.

Procedural Steps

1. Dilute sample 1:10 with Tripure or Trizol reagent (generally 50 µl of sample to 500 µl Tripure; or 100 µl sample to 1000 µl (1ml) Tripure) in clean screw cap microcentrifuge tube.
2. Mix well by inverting tube 10 times. To avoid aerosols, do not vortex or shake tube.
3. Incubate sample for at least 10 minutes at room temperature (20-25℃).
4. Transfer to a clean tube, then invert tube 10 times to inactivate the interior of the tube.
5. Clean the surface of the tube by dunking into a container of disinfectant, then wipe and put tube into a second leak-proof container before removing from the biosafety cabinet for transport or storing, as noted.

Notes:

- At this stage, samples are inactivated (non-infectious) and standard precautions for maintaining nucleic acids and minimizing cross-contamination should be followed.
- Samples can be stored for later RNA extraction procedure.
  - Samples should be stored at 2 to 8℃ for up to 6 hours.
  - Samples held from 6 hours to 2 weeks should be stored at -20℃.
  - Samples held for longer than 2 weeks should be stored at -80℃.
  - Frozen samples must not be thawed more than once. Repeated freezing and thawing leads to denaturation and precipitation of proteins, causing reduced yields of the isolated viral RNA.
- Circumventing the Tripure step and doing just the RNeasy is dangerous because the RLT buffer alone is not completely veridical.

B. Procedural steps for RNA Extraction (see Attachment F for procedure checklist)

WARNING: DO NOT CONTINUE UNLESS YOU HAVE INACTIVATED THE VIRUS USING THE RECOMMENDED TRIPURE OR TRIZOL REAGENTS DESCRIBED ABOVE.

DO NOT SKIP THE CRITICAL INACTIVATION STEP.

Notes-

- Perform all centrifugation steps at room temperature (20–25℃) in a standard microcentrifuge.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach or “RNase Zap™” or equivalent, to minimize risk of nucleic acid contamination.

1. Place inactivated samples in an appropriately ventilated workspace. This should be a designated space within the lab for sample manipulation. Allow frozen samples to thaw.
2. To the sample, add 200µL chloroform.

3. Vortex, then let sit for 5-15minutes (on ice, if possible), with frequent vortexing or vigorous shaking.
4. Centrifuge tube at 14,000 rpm for 15 minutes.
5. The mixture will separate into three phases:
   a. Colorless aqueous (upper) phase (RNA)
   b. White interphase (protein)
   c. Red organic (lower) phase (DNA)
   Note: For illustration, see Attachment E.

6. Remove the top (colorless) layer with a micropipettor and place in a new tube. ***(Note: Do not disturb white (protein) layer, as inclusion of protein will prevent further extraction of total RNA.)***
   ---
7. Add 1 volume of 70% ethanol
   Generally, 1 volume = the volume of Tripure Isolation Reagent (500µl per 100µl sample).
8. Mix well – DO NOT Centrifuge.
9. Add 700µl of the mixture to the RNEasy spin column. Include any precipitate that may have formed.
10. Spin at >10,000rpm for 15 seconds.
11. Discard flow-through. Wipe off drips with clean paper towel.
12. Concentrate the RNA by applying multiple aliquots of the same sample to a single column and repeating steps 9-11.
13. Add 700µl RW1 buffer.
14. Spin 15 seconds at room temperature.
15. Discard flow-through. Wipe off drips with clean paper towel.
16. Add 500µl RPE.
17. Spin 15 seconds.
18. Discard flow-through. Wipe off drips with clean paper towel.
19. Add 500µl RPE.
20. Spin 2 minutes.
21. Discard collection tube.
22. Put column in new collection tube and spin 1 minute.
23. Put column in a sterile, nuclease-free 1.5ml microcentrifuge tube for elution of RNA.
24. Add 50µl RNAse-free water to the column membrane.
    Note: Let sit at room temperature (20–25°C) for 1 minute to maximize elution.
25. Spin 1minute at room temperature. Purified RNA is found in the eluent which should be stored at -20°C to -80°C until tested.

The sample is now ready for molecular testing. (See SOP# VSPB1101: Ebola virus (Zaire) nucleoprotein Real Time Reverse Transcription Polymerase Chain Reaction (Real Time RT-PCR)

C. Clean up

1. Clean and decontaminate materials, equipment, and workspace. It is recommended to first clean with standard hospital detergent, followed by

decontamination with chlorine bleach, as organic matter can inactivate disinfectants.

2. Dispose of materials in biohazard waste bag or sharps container, in accordance with biosafety policies and the precautions described in Safety section of this SOP.

V. LIMITATIONS OF PROCEDURE

A minimum of 1:5 dilution (e.g. 200µl of serum placed into 1000µl of Tripure Isolation reagent or Trizol for a total of 1ml) with a GITC-containing buffer (e.g. Tripure Isolation Reagent) must be used to ensure complete inactivation of viral particles before removal from the containment laboratory.

It is important to pay close attention to technique and follow recommended protocols for designation of work areas, to avoid nucleic acid contamination.

VI. RECORDING

Record keeping is essential for good laboratory practice. Laboratory personnel are responsible for documenting completion of applicable sample inactivation and extraction procedural steps in appropriate logs and worksheets. See Attachments for examples of inactivation protocol check list and example worksheets.

VII. REFERENCES

Current Protocols in Molecular Biology, 2007. Available on-line through CDC's PHLIC.

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World Health Organization. Laboratory biosafety manual, 3rd Edition. Geneva: World Health Organization (WHO); 2004. Available from URL: http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf.

VIII. ATTACHMENTS

Attachment A: Checklist: Inactivation by Tripure/Trizol Lysis Buffers – Non-Tissues

Attachment B: Example Worksheet for Sample Inactivation

Attachment C: Checklist Using Qiagen's RNeasy Kit

Attachment D: How to Make Chlorine Solutions for Environmental Disinfection

Attachment E: Illustration of Separation Phases during Step # 5 of RNA Extraction Procedure

Attachment A:

Checklist: Inactivation by "Tripure/Trizol" Lysis Buffers – Non-Tissues (ex., blood, serum)

Date:_____ Location of work:_____

Laboratorian (print name):_____Email: _____
Patient Diagnosis (INFO):_____
Tests Requested_____
# of Specimens Inactivated:_____

Procedure	**Initial:**

☐ Add 0.5mL to 1.0mL of undiluted lysis reagent (Tripure or Trizol reagent) to clean microcentrifuge tubes

☐ Dilute specimen(s) in at least 10 volumes of lysis buffer (Tripure or Trizol) reagent (10:1 ratio). For example:

- 50uL of specimen + 500uL of lysis reagent
- 100uL of specimen + 1000uL of lysis reagent

☐ Mix well by vortexing, or invert tube 10 times

☐ Incubate specimen in lysis buffer for at least 10 minutes at room temperature

☐ Transfer contents to clean tube

☐ Vortex (or invert tube 10 times) to mix and to inactivate interior of tube

☐ Clean the surface of the tube by dunking into a container of disinfectant, then wipe and put tube into a second leak-proof transfer container before removing from the biosafety cabinet for testing.

☐ I certify that I have been trained to complete the above inactivation protocol, and will report to my supervisor if any further training or clarifications are needed to competently perform the above work.

Laboratorian Signature:_____

Attachment B: Example Worksheet for Sample Inactivation

Date Received	Sample ID	Patient Name	Test Requested	Date of Sample Inactivation	Time of Sample Inactivation	Tech Initials

Attachment C: Checklist Using Qiagen's RNeasy Kit

RNA EXTRACTION

WARNING: YOU MUST FIRST INACTIVATE VIRUS USING THE RECOMMENDED TRIPURE OR TRIZOL REAGENTS.

DO NOT SKIP THIS CRITICAL INACTIVATION STEP.

Adaptation of Qiagen's RNeasy kit for use with Tripure reagent

Using Qiagen's RNeasy kit:

☐ Add sample to Tripure for a 1:10 dilution (i.e, 100uL blood to 1000uL Tripure)

☐ Incubate 10 minutes at room temperature, transfer to new tube, disinfect the outside of tube

☐ Can freeze for later extraction (Do not keep at 4ºC for more than 6 hours)

☐ Add 200uL of Chloroform

☐ Vortex, incubate on ice if possible 5-15 minutes with frequent vortexing

☐ Spin 14,000 RPM, 15 minutes

☐ Pull off top layer being careful not to disturb middle (protein) layer and place in new tube

☐ Add 1 volume of 70% ethanol to the top layer, mix well

☐ Add 700uL to spin column, spin >10,000rpm for 15 seconds, discard flow-through (can repeat several times to concentrate the RNA)

☐ Add 700uL RW1 buffer, spin 15 seconds, discard flow-through

☐ Add 500uL RPE, spin 15 seconds, discard flow-through

☐ Add 500uL RPE, spin 2 minutes, discard collection tube

☐ Put column in new collection tube and spin 1 minute

☐ Put column in a new collection tube, add 30-50uL RNAse free water, incubate 1 minute

☐ Spin 1 minute (do not use the eluate again to try to get more RNA – you will lose RNA)

Attachment D: How to Make Chlorine Solutions for Environmental Disinfection

Example I - Using Liquid Bleach

Chlorine in liquid bleach comes in different concentrations. Any concentration can be used to make a dilute chlorine solution by applying the following formula:

$$\left[\frac{\text{\% chlorine in liquid bleach}}{\text{\% chlorine desired}}\right] - 1 = \text{Total parts of water for each part bleach†}$$

Example: To make a 0.5% chlorine solution from 3.5%‡ bleach:

$$\left[\frac{3.5\%}{0.5\%}\right] - 1 = 7 - 1 = 6 \text{ parts water for each part bleach}$$

Therefore, you must add 1 part 3.5% bleach to 6 parts water to make a 0.5% chlorine solution.

† "Parts" can be used for any unit of measure (e.g. ounce, litre or gallon) or any container used for measuring, such as a pitcher.

‡ In countries where French products are available, the amount of active chlorine is usually expressed in degrees chlorum. One degree chlorum is equivalent to 0.3% active chlorine.

Example II - Using Bleach Powder

If using bleach powder, † calculate the amount of bleach to be mixed with each litre of water by using the following formula:

$$\left[\frac{\text{\% chlorine desired}}{\text{\% chlorine in bleach powder}}\right] \times 1\,000 = \text{Grams of bleach powder for each litre of water}$$

Example: To make a 0.5% chlorine solution from calcium hypochlorite (bleach) powder containing 35% active chlorine:

$$\left[\frac{0.5\%}{35\%}\right] \times 1\,000 = 0.0143 \times 1\,000 = 14.3$$

Therefore, you must dissolve 14.3 grams of calcium hypochlorite (bleach) powder in each litre of water used to make a 0.5% chlorine solution.

† When bleach powder is used; the resulting chlorine solution is likely to be cloudy (milky).

Example III - Formula for Making a Dilute Solution from a Concentrated Solution

$$\text{Total Parts (TP) } (H_2O) = \left[\frac{\text{\% Concentrate}}{\text{\% Dilute}}\right] - 1$$

Example: To make a dilute solution (0.1%) from 5% concentrated solution.

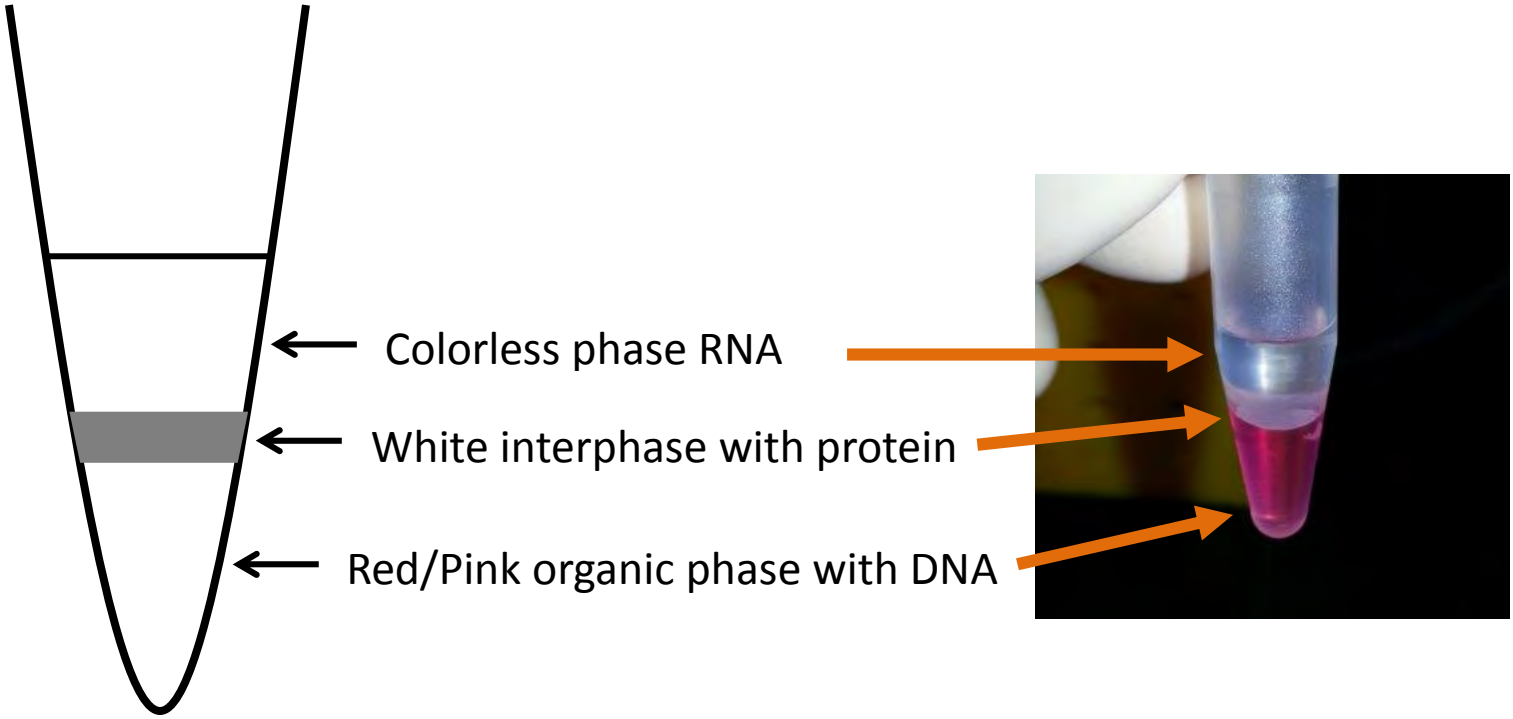
$$\text{Calculate TP } (H_2O) = \left[\frac{5.0\%}{0.1\%}\right] - 1 = 50 - 1 = 49$$

Take 1 part concentrated solution and add to 49 parts boiled (filtered if necessary) water.

Source:

AVSC International (1999). Infection Prevention Curriculum. Teacher's Manual. New York, p.267.

Attachment E: Illustration of Separation Phases during Step # 5 of RNA Extraction Procedure



Viral Special Pathogens Branch
Centers for Disease Control and Prevention

LABORATORY DIAGNOSTIC STANDARD OPERATING PROCEDURE

Facility:	
Title: Viral Special Pathogens Branch CDC - Ebola virus (Zaire) nucleoprotein Real Time Reverse Transcription Polymerase Chain Reaction (Real Time RT-PCR)	
Document Number: VSPB-1101	**Effective Date:** **Version Number:** 1
Other documents cross-referenced in this SOP: VSPB-1100: Inactivation of Enveloped RNA Viruses and Extraction of Genomic Material (RNA) using Tripure or Trizol Reagents and the Qiagen RNeasy Kit	

REVISION HISTORY

HISTORY OF CHANGES				
Rev. Level	Sections Changed	Description of Change (From—To)	Date	Approval
1		Adapted previous VSPB CDC protocols for international collaborators	08/20/2014	

I. PURPOSE

The Ebola virus (Zaire) nucleoprotein Real Time RT-PCR Protocol includes a primer and dual-labeled hydrolysis (Taqman®) probe set to be used in real-time PCR assays for the *in vitro* qualitative detection of viral RNA isolated from human and animal samples that include whole blood, serum, or plasma.

II. PRINCIPLE

Real-time polymerase chain reaction, also called quantitative real-time polymerase chain reaction (Q-PCR) is a laboratory technique based on the polymerase chain reaction (PCR), which is used to monitor the PCR amplification products in real time. A variation of Q-PCR is reverse transcription Q-PCR (Q-RT-PCR) which includes a reverse transcription step to create a complementary DNA strand from each single stranded RNA genome. In Q-PCR, the specific amplification product is detected via the release of fluorescent dyes during each amplification cycle. The fluorescent dyes are usually linked to oligonucleotides (called probes), which bind specifically to the amplified product. Monitoring the fluorescence intensities during the Q-PCR allows the detection and quantitation of the accumulating of product without having to open the reaction tubes after the PCR assay.

III. RESPONSIBILITIES

A. Staff Responsibilities

Position	Responsibility
Biosafety Officer	-Reviews this SOP and performs a risk assessment for the work areas intended to conduct it; -Reviews and approves designated locations for sample preparation, storage and testing activities for the site where each is to take place; Ensures staff is adequately trained in biosafety procedures.
Quality Officer	-Understands and enforces relevant certification information (agency or country-specific) as applicable to execution of this SOP. -Monitors adherence to this SOP by the personnel conducting it and oversees occurrence management where necessary.
Laboratory Manager	- Ensures appropriate protocols have been used and documented; - Ensures retention of applicable logs and worksheets; - Ensures testing personnel have the appropriate training.
Testing Personnel	- Adheres to all SOP instructions and biosafety requirements; - Documents completion of all procedural steps, as applicable.

B. Specific Safety and Containment Requirements and Responsibilities

This section specifies actions and considerations to be taken into account according to the risk assessment conducted by the Biosafety Officer, such as known hazards specific to this SOP, and biosafety level and practices required for the safe execution of its procedures and use of reagents. **Adherence to appropriate level safety guidelines and related biosafety SOPs is mandatory.**

IV. SAMPLE HANDLING

Samples addressed by this protocol include RNA extracted from the following human or animal samples: whole blood, sera, or plasma).

For information regarding extraction procedures, see VSPB-1100: Inactivation of Enveloped RNA Viruses and Extraction of Genomic Material (RNA) using Tripure or Trizol Reagents and the Qiagen RNeasy Kit.

V. REAGENTS - MATERIALS/SUPPLIES – EQUIPMENT

A. Materials/Supplies/Equipment for Biosafety

BIOSAFETY
Reagents
• Standard hospital detergent • Disinfectant (minimum of 0.5% sodium hypochlorite)*
Supplies
• Disposable, powder-free gloves • Laboratory coat • Additional PPE as needed, based on risk assessment • Biohazard waste bags/container • Sharps container • Disinfectant container • Absorbent towels
Equipment
• Class II Biosafety Cabinet, if available • Plexiglass shield or splashguards, at minimum

*Note-- **Chlorine solutions gradually lose strength, thus fresh solutions must be prepared daily.**

See Appendix C for instructions on how to prepare disinfectant ((minimum of 0.5% sodium hypochlorite)

B. Materials/Supplies/Equipment for Procedure

Molecular biology-grade reagents should be used and plastics should be sterile and certified RNAse/DNAse free.

Observe storage conditions and expiration dating as defined by the manufacturer.

PROCEDURE
Reagents

• Invitrogen's Superscript III Platinum® One Step qRT-PCR Kit (cat # 11732-020):
  o 2X PCR Master mix
  o RT/Platinum Taq Mix
  o Rox dye
• Molecular grade sterile nuclease free distilled water (RNase and DNase free) (Promega part no. P119C)
• RNaseOUTTM Recombinant Ribonuclease Inhibitor (Invitrogen cat # 10777-019)
• Forward primer: (50µM) ZaiNPTaqM1-F
• Reverse primer: (50µM) ZaiNPTaqM1-R
• Probe: (5µM) ZaiNPTaqMProbe 1
  o TaqMan® probes are labeled at the 5'-end with the reporter molecule (6-carboxyfluorescein [FAM] and with the quencher, Blackhole Quencher 1 [BHQ1] Biosearch Technologies, Inc., Novato, CA) at the 3'-end.
• Positive Control RNA isolated from primary or transformed cell culture supernatants or lysates
• Negative control RNA

Supplies
• Laboratory marking pen • Ice bucket containing ice for reaction or isolation tubes • 2µl, 10µl, 20µl, 200µl, and 1000µl adjustable pipettes and aerosol barrier tips • 0.2ml PCR reaction optical tube strips or plates (ABI part no. 4306737 or Eppendorf cat #'s 951010006 and/or 951020303) • Optical strip caps or optical sealing film (ABI part no. 4313663 or Eppendorf cat no. 951023035) • Sterile, nuclease free 2.0ml microcentrifuge tubes (ABI part no. 4305936)
Equipment
• Microcentrifuge • 96-well plate compatible centrifuge • Vortex • Real-time PCR detection system such as the Applied Biosystems 7500 or 7900HT Real-Time PCR System. • Applied Biosystems' Sequence Detection Software ver. 1.3.1 or 7900HT ver. 2.3 Sequence Detection Systems or similar software

C. Reagent Preparation

1. Frozen test reagents must be thawed and mixed by pipetting or by brief vortexing for 15 seconds followed by centrifugation at 6000 x g (8000 rpm) for 15 seconds; followed by placing the reagents in an ice bucket filled with ice to keep the labile components protected from excess temperatures.

   NOTE— Enzymes (i.e., Taq polymerase) are labile and should be kept frozen (-15 to -25 ºC) and not exposed to excessive freeze-thaw cycles. To avoid freeze-thaw cycling of enzymes, they should be stored separately in a cold block at -15 to -25 ºC.

2. For additional information on **Primers and Probes,** refer to Attachment A.

3. Some core reagents may be provided in bulk quantities. In such cases, these reagents need to be aliquotted before use to avoid excess freeze-thaw cycles in volumes appropriate to the laboratory workload. Label all aliquots with the following information:

   a. Name of component

   b. Concentration

   c. Date aliquotted

   d. Expiration

   e. Initials of person who prepared the aliquot

   f. Volume of aliquot

4. Use tough-tag or similar labels that are resistant to smudging in freezer conditions.

IV. QUALITY ASSURANCE/QUALITY CONTROL

Quality Assurance

An established quality assurance (QA) program should be in place in order to minimize the risk of errors and to maximize the possibility of discovering errors if they occur. The institutional QA program must follow the recommendations from established standards organizations for quality assurance.

- Prior to instituting this protocol, dedicated laboratory space which is physically segregated should be designated for each of the following activities and a risk assessment done of the work environment and processes. These include designated space for:
  - Specimen receiving, accession, and storage;
  - Sample inactivation and nucleic acid extraction;
  - Master mix preparation (clean space);
  - Reaction mixture and sample addition;
  - Amplification and detection.

- To avoid sample contamination:
  - Always wear powder-free gloves when handling reagents.
  - Never return an aliquot to the original container.
  - Maintain separate areas for assay setup.
  - Maintain separate areas for handling of nucleic acids.
  - Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., screw cap microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
  - Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
  - Never wear the same glove/gown at multiple stations.
  - Change gloves between samples whenever you suspect they may be contaminated.
  - Keep reagent and reaction tubes capped or covered as much as possible.

- General Equipment preparation:
  - Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach or "RNase Zap™" or equivalent, to minimize risk of nucleic acid contamination.

- At each step, verify that the integrity of all sample information is maintained. If clerical errors are discovered, correct and document by initialing. If the integrity of sample identity is compromised at any step, processing must be repeated for the affected steps of the analysis. Document the actions taken. Specimen identity must be documented and verified at every step as part of standard operating procedure.

- Record the lot/batch number of reagents and their expiration dates in applicable PCR worksheets and logs (see example in the Attachment B).
- All PCR reagents should be stored according to manufacturer recommendations. **Do not use reagents after they have expired**.

Quality Control

- Quality control materials consist of:
  - Positive Control (RNA isolated from cell cultures infected with known virus)
  - Negative control
  - No template controls (NTC)) – Contains reaction mix (buffers, primers, probes) but no RNA.
  - Suitable housekeeping gene (e.g. 18S RNA) - should be included in conjunction with any sample extracted from a clinical specimen to ensure the quality of the RNA extraction procedure
  - Mock extraction controls (MOCK) - provides a secondary negative control that validates the nucleic extraction procedure, reagent

integrity, and non-reactivity of the sample matrix (cell culture, serum, etc.)

- Control materials should be included in each run.

- Reactions should be prepared so that the Negative Control is set up first, then unknown samples, followed by the Positive Control, and lastly, another Negative Control. Preparation in this order will reveal whether poor technique may have resulted in positive samples contaminating the Negative Control and other unknown samples.

- If controls do not perform as expected, the assay should be repeated. A discordant control result may indicate a procedural error.
  DO NOT report test results until problems have been resolved.

V. PROCEDURE

A. Preparation of Equipment and Laboratory Work Area

Notes:
- All work tasks in the Molecular Laboratory are restricted to work zones which are based on the potential to spread contamination (see section on Quality Assurance).
- The preparation of the Master Mix should be conducted within a PCR workstation.

1. Use appropriate personal protective equipment (PPE), according to risk assessment and as recommended in the Safety section of this SOP.

2. Prepare disinfectant (minimum of 0.5% sodium hypochlorite) and add 100 ml of disinfectant to the disinfectant container.

3. Clean the work area where the tests will be performed with disinfectant.

B. Reagent Set-up

NOTE: Keep all reagents on ice during assay set up.

Primers and probes
1. Thaw frozen aliquots of primer and probes.
2. Vortex all primers and probes to ensure uniform mixing.
3. Briefly centrifuge all primers and probes and then place on ice.

RT-PCR reagents
1. Thaw master mix on ice.
2. After thawing, mix by inversion.

3. Briefly centrifuge master mix and enzyme, and then place back on ice.

Reaction Assay Mixtures

Reaction assay mixtures are made as a cocktail in a separate clean room and dispensed into the reaction wells. Water, extracted nucleic acid, or positive template controls are then added to the appropriate test reactions and controls.

1. Label one 1.5 ml microcentrifuge tube for preparation of the master mix.
2. Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction cocktail to allow for pipette carry over. See below:
    a. If number of samples (n) including controls = 1 to 14, then N = n + 1
    b. If number of samples (n) including controls > 15, then N = n + 2
3. **Master Mix:** Calculate the amount of each reagent to be added for each primer/probe set reaction master mix. The calculations are as follows:

Reagent	Amount ABI7500	Amount ABI7900
2X PCR Master Mix	N x 25µl	N x 25µl
RT/Platinum Taq Mix	N x 1µl	N x 1µl
Forward primer (50µM stock)	N x 1µl	N x 1µl
Reverse primer (50µM stock)	N x 1µl	N x 1µl
Probe (5µM stock)	N x 1µl	N x 1µl
*** ROX ***	N x 0.1µl	N x 1.0µl
*** Nuclease free water ***	N x 15.9	N x 15µl
Total volume	N x 45.0µl	N x 45.0µl

ROX = use 0.1µl for reactions run on the ABI7500 or 1µl for the ABI7900, and q.s. with Nuclease free water (15.7µl for 7500 or 14.8µl for 7900)

C. Procedural steps

1. In the assay set up area:
    a. Dispense reagents in accordance with above table into labeled 1.5ml microcentrifuge tubes.
    b. After final addition of the water, mix reaction mixtures by pipetting up and down. **Do not vortex**.
    c. Centrifuge for 5 seconds to collect contents at bottom of the tube, and then place the tube on ice.
    d. Set up reaction strip tubes or plates in 96-well cooler rack or on ice.
    e. Dispense 45µl of each master mix into each well.
    f. Before moving the plate to the nucleic acid handling area, set up the no template control (NTC) reactions in the assay set-up area by pipetting 5µl of nuclease free water into the NTC wells.

g. Cover the reaction plate with optical adhesive cover and move the reaction plate to the nucleic acid handling area.
h. Waste should be removed from the lab daily.

2. In the nucleic acid handling area:

a. Vortex the tubes containing the samples for 5 seconds.
b. Centrifuge tubes for 5 seconds.
c. Pipette 5µl of the sample into the appropriate well for that sample. Repeat for the remaining samples.
d. Note: Change tips after each reagent addition, and cap the tubes at logical intervals or as soon as possible after sample RNA addition (e.g. end of a row).
e. Add 5µl of mock extracted sample to the MOCK wells. Cap MOCK wells.
f. Pipette 5µl of human positive control RNA into the appropriate wells. Cap wells.
g. Label:

If using 8-tube strips:

1) Label the TAB of each strip to indicate sample position (DO NOT LABEL THE TOPS OF THE REACTION TUBES!).
2) Briefly centrifuge tube strips for 10-15 seconds. Return strip tubes to cold rack/ice.

If using plates:

3) Label the edge of the plate such that the label does not cover any of the wells.
4) Centrifuge at 500 x g for 30 seconds at 4°C. Return to cold rack/ice.

3. Amplification:

The reaction volume is 50µl.

*Fluorescence data for test fluorochromes (FAM) and reference standard (ROX) should be collected during the 55°C incubation step. Use the detector program named ZEBOV-NPdx with the following condition:

	Conditions
Reverse transcription	50°C for 15 min
Taq inhibitor inactivation	95°C for 2 min
PCR amplification (40 cycles)	95°C for 15 sec 55°C for 1 min*

4. Post-Amplification:

The expected product size is 79bp.

a. Open the diagnostic run file using suitable software (e.g. ABI's Sequence Detection Software ver. 1.3.1).
   1) Go to the Results tab, and then the Amplification plot tab
   2) Click "Auto Ct" on the right hand side, and then click the Analyze button below.
   3) Export the results (under the File menu) and note the Threshold level that was set automatically during the analysis.

A. Clean up

Do Not Open Tubers or PCR Plates!

1. Clean and decontaminate materials, equipment, and workspace. It is recommended to first clean with standard hospital detergent, followed by decontamination with chlorine bleach, as organic matter can inactivate disinfectants.
2. Dispose of materials in biohazard waste bag or sharps container, in accordance with biosafety policies and the precautions described in Safety section of this SOP.

V. INTERPRETATION OF RESULTS

1. Evaluate controls for acceptability:
   a. All positive samples should exhibit amplification curves with Ct values that cross the threshold line at or before 40 cycles, thus indicating the presence of RNA and that the specimen is of acceptable quality.
   If the positive RNA control fails to exhibit CT values that cross the threshold at or before 40 cycles the assay is invalid and must be repeated. Failure to detect ZEBOV-NP in any of the positive samples may indicate:
      1) Improper extraction of nucleic acid from samples resulting in loss of RNA or carry-over of inhibitors from specimens.
      2) Absence of sufficient RNA to enable detection.
      3) RNA degradation due to poor specimen shipping conditions or handling procedures.
      4) Improper assay set up and execution.
      5) Reagent or equipment malfunction.

   b. The NTC reactions and mock extraction controls for probe/primer sets should not exhibit fluorescence curves that cross the threshold line before 40 cycles. If a false positive occurs with one or more of the primer and probe NTC reactions, sample contamination may have occurred. Invalidate the run and repeat the assay with stricter adherence to the procedure guidelines.

2. Unknown Specimen Interpretation:

a. In order for a test run to be valid, all NTC reactions must be negative without an amplification curve. If one or more NTC fails, the entire run is invalid and potential sources of contamination should be identified and corrected. Re-test the purified specimen and controls, and re-analyze. Results should NOT be reported until all false-positive NTC reactions are corrected.
b. The external negative processing control associated with each extraction batch should be negative with no amplification curves. If the external sample processing negative control for a set of extracted specimens is positive, all results for those specimens are invalid. Re-test the purified specimen and controls and re-analyze. If the re-test for the processing control results is positive, then re-extract the specimens and controls. Results should NOT be reported until all false-positive negative processing control reactions are corrected.
c. A specimen is considered positive for Ebola virus (Zaire) if the specimen has a positive amplification result using this assay, if the above conditions are met.
d. A specimen is considered negative for Ebola virus (Zaire) if the assay does not have a positive amplification result and the internal reaction control (i.e., 18s rRNA or B2 Microglobin if whole blood) assay has a positive amplification result. If the specimen is serum, these internal controls may not be valid. Please contact the CDC via email at EOCEVENT246@CDC.GOV for additional guidance.

VI. LIMITATIONS OF PROCEDURE

- This protocol was optimized using Invitrogen's Superscript III Platinum® One Step RT-PCR Kit and both the Applied Biosystems 7500 and 7900HT Real-Time PCR Systems. It has not been validated with other reagent kits or thermocyclers, but would be expected to perform similarly with similar reaction conditions and other settings.

- Performance of PCR amplification-based assays depends on the amount and quality of sample template RNA. See RNA extraction protocol MD1001 for further details.

VII. RECORDING / REPORTING

Controls and Test results must be recorded on the applicable worksheet or log, and reported as designated by facility policies.

VIII. REFERENCES

Current Protocols in Molecular Biology. 2007. Available on-line through CDC's PHLIC.

Mifflin, TE. Setting Up a PCR Laboratory. Mifflin TE. Setting up a PCR Laboratory. In: Newton CR, editor. PCR: Essential Data. New York: John Willey and Sons Inc; 1995. p. 5-14.

Sambrook, J., E.F. Fritsch, T. Maniatis. 1989. Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 6.2-6.7.

Towner, J, T. Sealy, T. Ksiazek and S. Nichol. (2007) High-Throughput Molecular Detection of Hemorrhagic Fever Virus Threats with Applications for Outbreak Settings. J Infect Dis. 2007 Nov 15;196 Suppl 2:S205-12

IX. ATTACHMENTS

Attachment A: Primer and Probe Sets
Attachment B: Example of PCR worksheet for samples and result records
Attachment C: How to Make Chlorine Solutions for Environmental Disinfection

Attachment A: Primer and Probe Sets

Primers/Probes	Sequence (5'>3')	Working Conc.	Lot No.
ZaiNPTaqM1-F	TGG AAA AAA CAT TAA GAG AAC ACT TGC	50µM	YYYYY
ZaiNPTaqM1-R	AGG AGA GAA ACT GAC CGG CAT	50µM	YYYYY
ZaiNPTaqMProbe 1*	CA TGC CGG AAG AGG AGA CAA CTG AAG C	5µM	YYYYY

*TaqMan® probes are labeled at the 5'-end with the reporter molecule 6-carboxyfluorescein (FAM) and with the quencher, Blackhole Quencher 1 (BHQ1) (Biosearch Technologies, Inc., Novato, CA) at the 3'-end.

Attachment B: Example of PCR worksheet for samples and result records

Sample Accession Number	Capillary	Crossing Point (Cp) value or Cycling Threshold (Ct)	Results	
			Qualitative (Positive / Negative)	Quantitative
	1			
	2			
	3			
	4			
	5			

Laboratorian (name):

Date DNA/RNA Isolated:

Method of DNA/RNA Isolation:

PCR Kit Lot #:

Kit Expiration Date of LC PCR test:

Attachment C: How to Make Chlorine Solutions for Environmental Disinfection

Example I - Using Liquid Bleach

Chlorine in liquid bleach comes in different concentrations. Any concentration can be used to make a dilute chlorine solution by applying the following formula:

$$\left[\frac{\%\text{ chlorine in liquid bleach}}{\%\text{ chlorine desired}}\right] - 1 = \text{Total parts of water for each part bleach}\dagger$$

Example: To make a 0.5% chlorine solution from 3.5%‡ bleach:

$$\left[\frac{3.5\%}{0.5\%}\right] - 1 = 7 - 1 = 6 \text{ parts water for each part bleach}$$

Therefore, you must add 1 part 3.5% bleach to 6 parts water to make a 0.5% chlorine solution.

† "Parts" can be used for any unit of measure (e.g. ounce, litre or gallon) or any container used for measuring, such as a pitcher.

‡ In countries where French products are available, the amount of active chlorine is usually expressed in degrees chlorum. One degree chlorum is equivalent to 0.3% active chlorine.

Example II - Using Bleach Powder

If using bleach powder, † calculate the amount of bleach to be mixed with each litre of water by using the following formula:

$$\left[\frac{\%\text{ chlorine desired}}{\%\text{ chlorine in bleach powder}}\right] \times 1\,000 = \text{Grams of bleach powder for each litre of water}$$

Example: To make a 0.5% chlorine solution from calcium hypochlorite (bleach) powder containing 35% active chlorine:

$$\left[\frac{0.5\%}{35\%}\right] \times 1\,000 = 0.0143 \times 1\,000 = 14.3$$

Therefore, you must dissolve 14.3 grams of calcium hypochlorite (bleach) powder in each litre of water used to make a 0.5% chlorine solution.

† When bleach powder is used; the resulting chlorine solution is likely to be cloudy (milky).

Example III - Formula for Making a Dilute Solution from a Concentrated Solution

$$\text{Total Parts (TP) } (H_2O) = \left[\frac{\%\text{ Concentrate}}{\%\text{ Dilute}}\right] - 1$$

Example: To make a dilute solution (0.1%) from 5% concentrated solution.

$$\text{Calculate TP } (H_2O) = \left[\frac{5.0\%}{0.1\%}\right] - 1 = 50 - 1 = 49$$

Take 1 part concentrated solution and add to 49 parts boiled (filtered if necessary) water.

Source:

AVSC International (1999). Infection Prevention Curriculum. Teacher's Manual. New York, p.267.

CENTERS FOR DISEASE CONTROL AND PREVENTION

DEPARTMENT OF HEALTH AND HUMAN SERVICES

UNITED STATES OF AMERICA

Instructions for Shipping Specimens to CDC

RELEASE TO THE PUBLIC

VERSION 1.0

AUGUST 2014

Frequently Asked Questions regarding Ebola Virus Specimen Collection and Shipment for International Laboratories

Centers for Disease Control and Prevention
1600 Clifton Road, Atlanta Ga, 30333
Emergency Operations Center (EOC)
2014 Ebola Virus Laboratory Team
+1 (770) 488-7100
Eocevent246@cdc.gov

Notification and Consultation

Hospitals should follow their national and local guidance for notification and consultation for Ebola testing requests first. CDC may then be contacted in consultation with your Ministry of Health IHR point of contact and WHO to request Ebola virus testing.

CDC cannot accept any specimens without prior consultation. For consultation, call the Emergency Operations Center at +1-770-488-7100.

When Specimens Should Be Collected for Ebola Testing

Ideally, specimens should be taken when a symptomatic patient reports to a health care facility and is suspected of having an Ebola virus exposure. However, if the onset of symptoms is less than three days, a subsequent specimen will be required to rule out Ebola.

- Patients can transmit the virus once symptoms appear and through the later stages of disease, as well as postmortem.
- Ebola virus can be detected in blood **only** after the onset of symptoms, most notably fever.
- It may take up to three days after the onset of symptoms for the virus to reach detectable levels.
- Virus is generally detectable by real-time RT-PCR from 3-10 days after the onset of symptoms.

Preferred Specimens for Ebola Testing

Specimens should include a minimum volume of 4 milliliters whole blood preserved with any of the following:

- EDTA, or clot activator, sodium polyethanol sulfonate (SPS), or Citrate.

Submit specimens for Ebola virus disease testing in plastic collection tubes. Do **not** submit specimens to CDC in glass containers. Do **not** submit specimens preserved in heparin tubes.

Specimens should be stored and shipped at 4°C or frozen on ice packs or on dry ice.

Note: Specimens other than blood (i.e. skin snips from post mortem) may be submitted after consulting with the CDC. For a consultation, call the Emergency Operations Center at +1-770-488-7100.

Apply standard labeling for each specimen. Identify the requested test on the requisition and CDC specimen submission forms. These forms are located later in this information packet in the "Shipping Information" section.

Diagnostic Testing for Ebola Performed at CDC

CDC is a WHO recognized Collaborating Center for diagnosis of Viral Hemorrhagic Fevers. Several diagnostic tests are available for detection of Ebola virus disease.

- Acute infections will be confirmed using a real-time RT-PCR assay (CDC test directory code CDC -10309 Ebola Identification).
- Virus isolation may also be attempted.
- Serologic testing for IgM and IgG antibodies will be completed for certain specimens and to monitor the immune response in confirmed Ebola virus disease patients (#CDC-10310 Ebola Serology).

Lassa fever is also endemic in certain areas of West Africa and may show symptoms similar to early Ebola virus disease. Diagnostic testing to identify Lassa fever can also be done at CDC.

- Diagnostic tests including but not limited to RT-PCR, antigen detection, and IgM serology may be used to rule out Lassa fever in Ebola virus disease-negative patients.

Transporting Specimens within the Hospital / Institution

In compliance with 29 CFR 1910.1030, place specimens in a durable, leak-proof secondary container for transport within a facility.

Important: To reduce the risk of breakage or leaks, **do not use any pneumatic tube system** for transporting suspected Ebola virus disease specimens.

Packaging and Shipping Clinical Specimens to CDC

Do not open collection tubes or aliquot specimens collected for Ebola virus disease testing.

Package specimens in their collection tubes for shipment following the basic triple packaging system:

- a primary receptacle (a sealable specimen bag) wrapped with absorbent material,
- placed inside a secondary receptacle (watertight, leak-proof), and then
- placed in turn in an outer shipping package (see IATA drawing below)

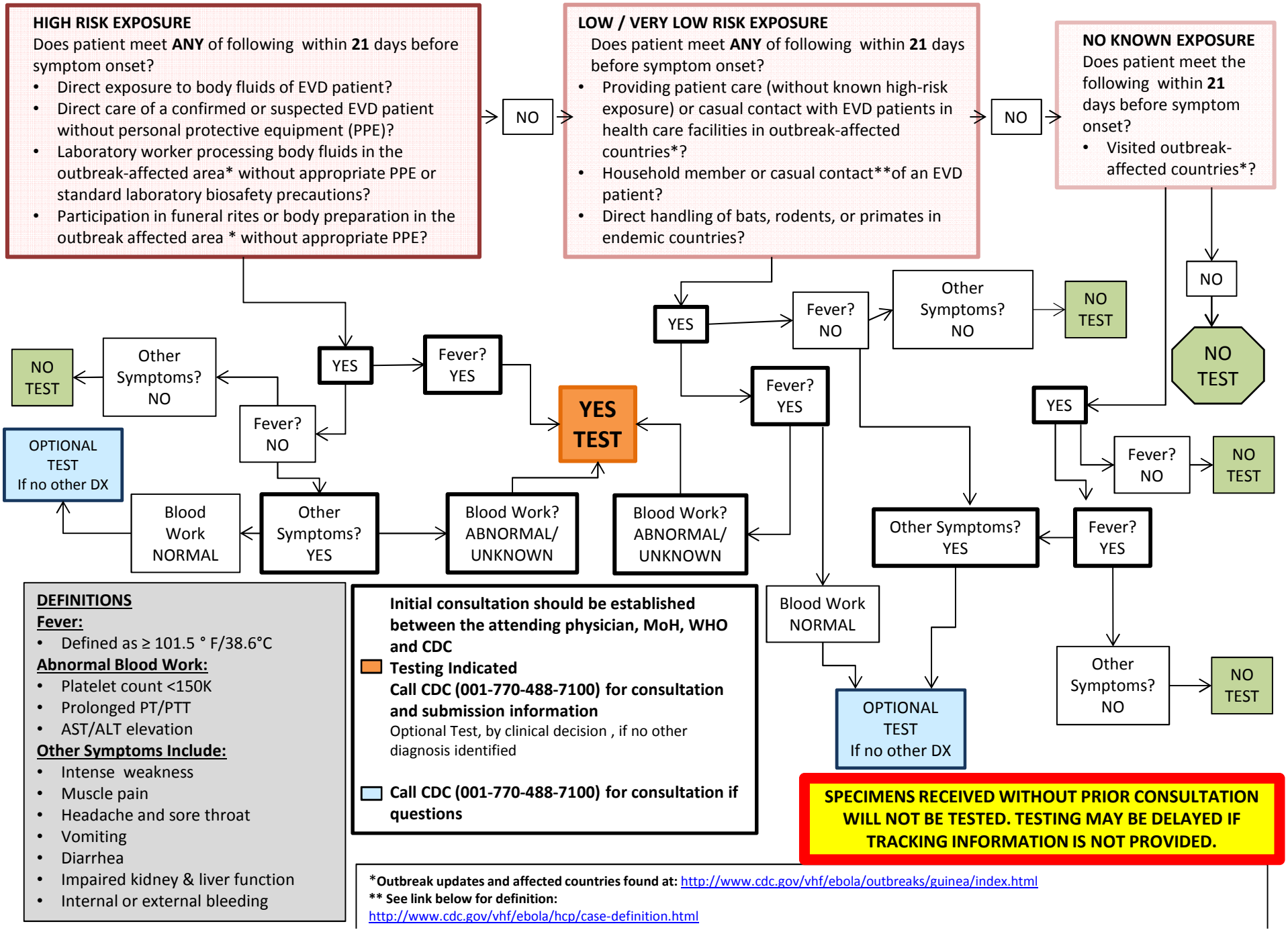
The Submission Process

Contact your Ministry of Health IHR point of contact and WHO, in consultation with CDC, to determine the proper category for shipment and packaging requirements. Packaging requirements should be based on clinical history and assessment of patient by the local Ministry of Health, WHO and CDC. A United States Public Health Service Import permit will be supplied by CDC if testing and shipment of specimens to CDC is required.

Note: National guidelines may differ; consult your country's guidelines before shipping.

Information on shipping and tracking is available at www.cdc.gov/ebola.

DECISION GUIDE FOR CDC CONSULTATION AND DIAGNOSTIC EBOLA VIRUS DISEASE (EVD) TESTING



DEFINITIONS

Fever:
- Defined as ≥ 101.5 ° F/38.6°C

Abnormal Blood Work:
- Platelet count <150K
- Prolonged PT/PTT
- AST/ALT elevation

Other Symptoms Include:
- Intense weakness
- Muscle pain
- Headache and sore throat
- Vomiting
- Diarrhea
- Impaired kidney & liver function
- Internal or external bleeding

Initial consultation should be established between the attending physician, MoH, WHO and CDC

Testing Indicated
Call CDC (001-770-488-7100) for consultation and submission information
Optional Test, by clinical decision , if no other diagnosis identified

Call CDC (001-770-488-7100) for consultation if questions

SPECIMENS RECEIVED WITHOUT PRIOR CONSULTATION WILL NOT BE TESTED. TESTING MAY BE DELAYED IF TRACKING INFORMATION IS NOT PROVIDED.

***Outbreak updates and affected countries found at:** http://www.cdc.gov/vhf/ebola/outbreaks/guinea/index.html
****See link below for definition:**
http://www.cdc.gov/vhf/ebola/hcp/case-definition.html

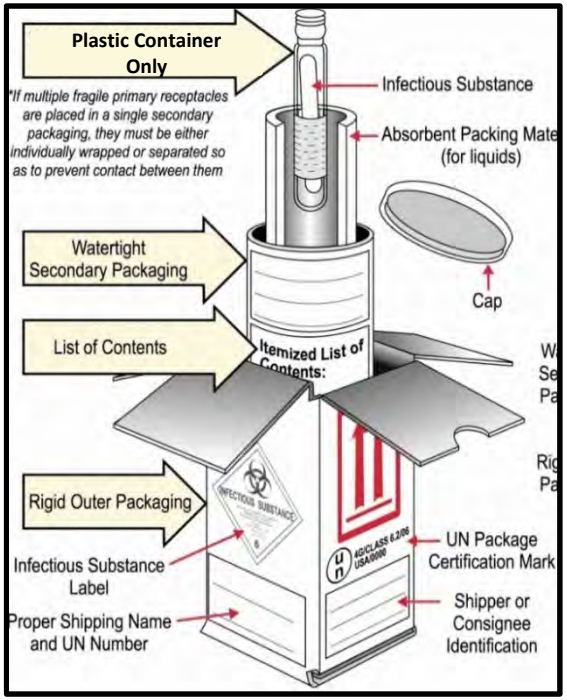
Decision to Test or Ship
Consultation between Clinician, MoH, WHO and CDC

SPECIMENS RECEIVED WITHOUT PRIOR CONSULTATION WILL NOT BE TESTED. TESTING MAY BE DELAYED IF TRACKING INFORMATION IS NOT PROVIDED.

Contact CDC EOC
For Shipping Instructions

CDC Consultation
See Documents
Shipment Checklist
Hospital Checklist

Complete IATA Form and Relevant
CDC/VSPB Specimen Forms
PHS Permit
Acceptance Checklist
IATA Fillable



IATA Compliant Triple Package
Category A Style
Category A Packing
Packaging Checklist

Send Tracking Information to CDC EOC

**** NO SPECIMENS ACCEPTED WITHOUT PRIOR CONSULTATION ****

Call (404) 639-1510 or (404) 639-1115 for authorization to ship specimens.

Instructions for submitting Diagnostic Specimens to CDC's Viral Special Pathogens Branch

1 **Label all samples with the following information: Patient's name or ID number, specimen ID number, date of collection**

For PCR/virus isolation, submit:

- Preferred: whole blood (purple, yellow, or blue top tube), fresh frozen tissue. Serum can also be used if only sample available.
- Minimum sample volume: 4 mL
- Fresh frozen tissues should be at least 1 cm^3, except for biopsies.
- Please ship sample frozen on dry ice in a plastic tube. Do not freeze glass tubes.

For serologic testing, submit:

- Serum (red top tube or serum separator)

-- or --

- Whole blood (purple, green, or blue top tube)
- Minimum sample volume: 4 mL
- Please ship sample refrigerated or frozen on ice packs.

Immunohistochemistry, submit:

- Formalin-fixed or paraffin-embedded tissues may be submitted:
  Preferred: lung, kidney, liver, spleen
  Other tissues can be submitted if available.
- Paraffin blocks are preferred, particularly if death was not recent.
- Ship paraffin blocks or formalin-fixed tissue at room temperature. Do not freeze.
- An autopsy or surgical report must accompany the specimen.

The following forms should be completed for each patient:

- CDC Specimen Submission Form
- VSPB Diagnostic Specimen Submission Form, on following page.
- For Hantavirus Pulmonary Syndrome testing, also submit the HPS Case Report Form
- Include a copy of all above Forms with the specimens.

Specimen packaging requirements:

- Please contact your state health department for approval to submit a specimen to CDC for laboratory testing.
- Contact your state and/or local health department and CDC to determine the proper category for shipment based on clinical history and risk assessment by CDC. State guidelines may differ and state or local health departments should be consulted prior to shipping.
- Package in accordance with the International Air Transport Association, regulations to prevent leakage. (See https://www.iata.org/publications/dgr/Pages/manuals.aspx and http://www.hercenter.org/regsandstandards/Transporting_Infectious_Substances_Safely.pdf)
- Include the following information on the Diagnostic Specimen Submission Form: your name, the patient's name, test(s) requested, date of collection, laboratory or accession number, and the type of specimen being shipped.
- On the outside of the box, specify how the specimen should be stored: **Frozen, Refrigerated**, or **Do Not Refrigerate**.
- Send specimens by overnight courier. International submitters should consider door-to-door shipment via air transport to expedite specimen delivery to CDC.
- Be sure to check '**Saturday Delivery**' if desired.
- Email the tracking number to the Viral Special Pathogens Branch.

5

HOW TO SUBMIT THE SPECIMENS AND FORMS TO CDC

Specimen submission address (if approved by state):	**Form submission by email:**	**Form submission by fax:**
Centers for Disease Control and Prevention ATTN STAT LAB: VSPB, Unit #70 1600 Clifton Road NE Atlanta, GA 30333 Phone: (404) 639-1115	Hit the 'Send to CDC' button at the bottom right of page 2. Your computer will generate an email containing the completed information. Hit the 'Send' button in your email application to send the email to CDC. Acknowledgement of receipt is not provided.	(404) 639-1118 or (404) 639-1509

**** NO SPECIMENS ACCEPTED WITHOUT PRIOR CONSULTATION ****

Call (404) 639-1510 or (404) 639-1115 for authorization to ship specimens.

Viral Special Pathogens Branch Diagnostic Specimen Submission Form

• Hantavirus Pulmonary Syndrome (HPS)* and other hantaviruses	☐	• Tick-borne Encephalitis	☐
• Ebola HF*	☐	• Lymphocytic choriomeningitis (LCM)	☐
• Marburg HF*	☐	• Hemorrhagic Fever with Renal Syndrome (HFRS)	☐
• Lassa Fever*	☐	• Rift Valley Fever	☐
• Crimean-Congo hemorrhagic fever (CCHF)*	☐	• Other hemorrhagic fevers: _____	☐

PLEASE COMPLETE ONE FORM PER PATIENT

** indicates a Notifiable Disease*

*** Please check off boxes to indicate testing request(s).***

PATIENT NAME:	Patient ID no.:
DOB:	DATE OF SYMPTOM ONSET:
CLINICAL DESCRIPTION:	

No.	Specimen ID No.	State Lab ID No.	Date collected	Specimen type
1				
2				
3				
4				
5				

FOR STATE HEALTH DEPARTMENTS	
Report/send results to: Person's name: Affiliation:	Phone no., fax no., and email address:
State Health Lab:	Phone no. and email address:
Person shipping specimen(s): Affiliation:	Phone no. and email address:
Physician's name: Affiliation:	Phone no. and email address:
State health department contact:	Phone no. and email address:
	Airway bill # (if known):

Instructions: You must have internet access and an email address to submit this Form electronically. Upon hitting the 'Send to CDC' button, a PDF is created, attached to an email, which you should then send to the address which appears in the address header; you may also cc: others. Acknowledgement of receipt by CDC is not provided. To print this form in order to fax or mail it, be sure to Save this form first.

Submit by Email

For hantavirus/HPS, be sure to provide a copy of this Form - to your state Health Department. -

SHIPPER'S DECLARATION FOR DANGEROUS GOODS

Shipper

XXXXXXXXXXXXXXXX

Air Waybill No.

Page 1 of 1 Pages

Shipper's Reference Number *(optional)*

Consignee

Dr Rollin, Viral Special Pathogens branch
MS G-14, CDC
1600 Clifton Rd, Atlanta, GA 30329, USA
Tel. 404-639-1115

For optional use for Company logo name and address

Two completed and signed copies of this Declaration must be handed to the operator.

WARNING

Failure to comply in all respects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties.

TRANSPORT DETAILS

This shipment is within the limitations prescribed for: *(delete non-applicable)*

PASSENGER AND CARGO AIRCRAFT | CARGO AIRCRAFT ONLY

Airport of Departure: XXXXXXXXXXXXXXX

Airport of Destination: Atlanta,GA USA

Shipment type: *(delete non-applicable)*

NON-RADIOACTIVE | ~~RADIOACTIVE~~

NATURE AND QUANTITY OF DANGEROUS GOODS

Dangerous Goods Identification				Quantity and type of packing	Packing Inst.	Authorization
UN or ID No.	Proper Shipping Name	Class or Division (Subsidiary Risk)	Packing Group			
UN 2814	Infectious substance, affecting humans (Suspected Category A Infectious Substance)	6.2		1 fiberboard box x 25 mL	620	
UN 1845	DRY ICE	9	III	____ KG Overpack used	954	

Additional Handling Information

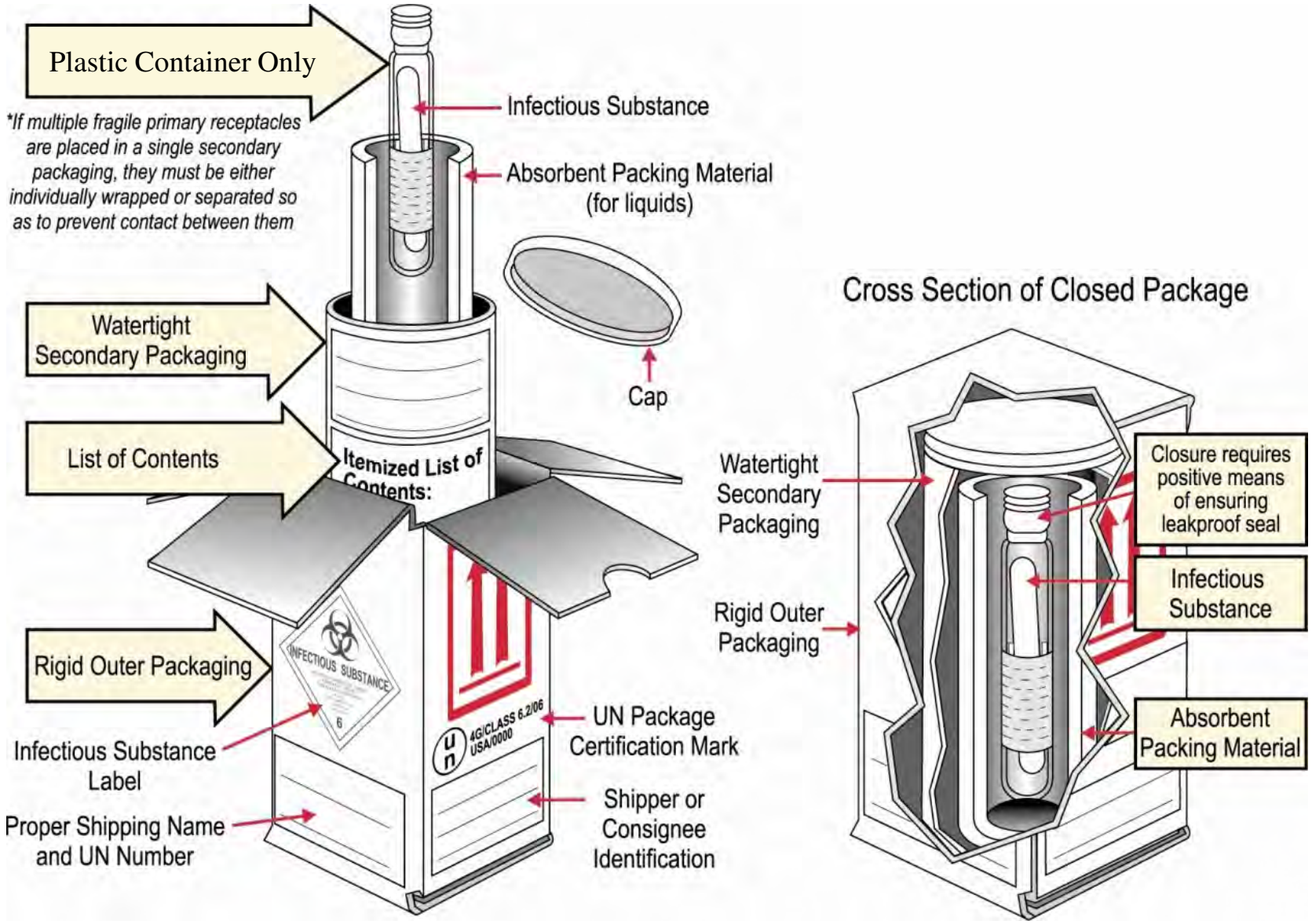
Specimen to be kept at + 4 C.
Responsible person: Dr. Rollin 404-639-1115. Atlanta,GA USA

I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name, and are classified, packaged, marked and labelled/placarded, and are in all respects in proper condition for transport according to applicable international and national governmental regulations. I declare that all of the applicable air transport requirements have been met.

Name/Title of Signatory
XXXXXXXXXXXXXXX

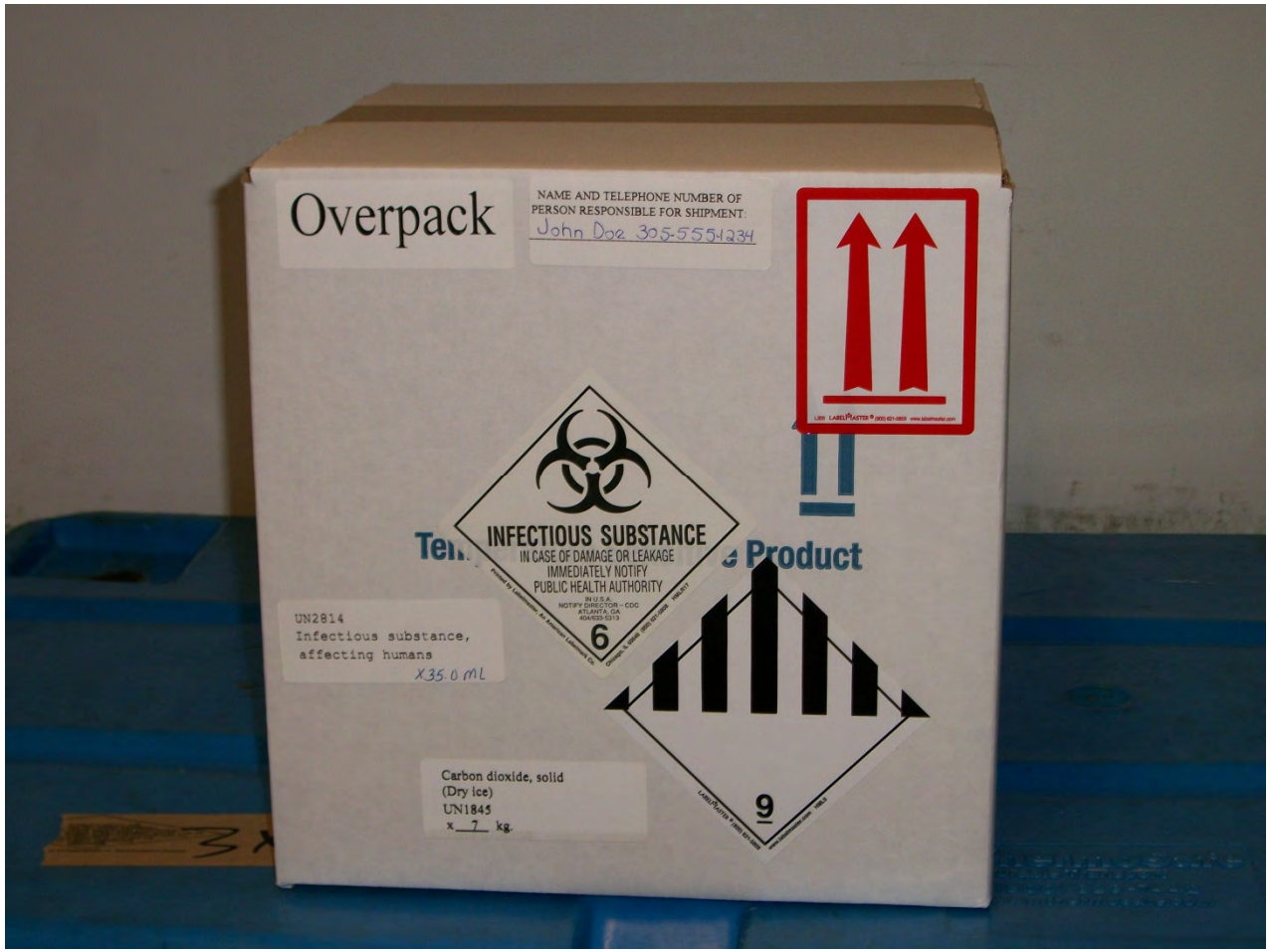
Place and Date
XXXXXXXXXXXXXXXXXXXXXXXXX

Signature
(see warning above)


Plastic Container Only
*If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated so as to prevent contact between them
Infectious Substance
Absorbent Packing Material (for liquids)
Watertight Secondary Packaging
Cap
List of Contents
Itemized List of Contents:
Rigid Outer Packaging
INFECTIOUS SUBSTANCE
6
4G/CLASS 6.2/06 USA/0000
UN Package Certification Mark
Infectious Substance Label
Proper Shipping Name and UN Number
Shipper or Consignee Identification
Cross Section of Closed Package
Watertight Secondary Packaging
Rigid Outer Packaging
Closure requires positive means of ensuring leakproof seal
Infectious Substance
Absorbent Packing Material

IATA Packing Instructions 620 & 954

Overpack Markings and Labels



2014
ACCEPTANCE CHECKLIST FOR DRY ICE (Carbon Dioxide, solid)
(For use when a Shipper's Declaration for Dangerous Goods is not required)

A checklist is required for all shipments of dangerous goods (9.1.4) to enable proper acceptance checks to be made. The following example checklist is provided to assist shippers and carriers with the acceptance of dry ice when packaged on its own or with non-dangerous goods.

Is the following information correct for each entry?

DOCUMENTATION

	YES	NO*	N/A
The Air Waybill contains the following information in the "Nature and Quantity of Goods" box (8.2.3)			
1. The UN Number "1845", preceded by the prefix "UN"	☐	☐	
2. The words "Carbon dioxide, solid" or "Dry ice"	☐	☐	
3. The number of packages of dry ice (may be in the pieces field of the AWB when they are the only packages in the consignment)	☐	☐	
4. The net quantity of dry ice in kilograms	☐	☐	
Note: *The packing instruction "954" is optional.*			
Quantity			
5. The quantity of dry ice per package is 200 kg or less [4.2]	☐	☐	
PACKAGES AND OVERPACKS			
6. The number of packages containing dry ice delivered as shown on the Air Waybill	☐	☐	
7. Packages are free from damage and in a proper condition for carriage	☐	☐	
8. The packaging conforms with Packing Instruction 954 and the package is vented to permit the release of gas	☐	☐	
Markings & Labels			
9. The UN number "1845" preceded by prefix "UN" [7.1.4.1(a)]	☐	☐	
10. The words "Carbon dioxide, solid" or "Dry ice" [7.1.4.1(a)]	☐	☐	
11. Full name and address of the shipper and consignee [7.1.4.1(b)]	☐	☐	
12. The net quantity of dry ice within each package [7.1.4.1(d)]	☐	☐	
13. Class 9 label affixed [7.2.3.9]	☐	☐	
14. Irrelevant marks and labels removed or obliterated [7.1.1(b); 7.2.1(a)]	☐	☐	☐
Note: *The Marking and labelling requirements do not apply to ULDs containing dry ice*			
For Overpacks			
15. Packaging Use markings and hazard and handling labels, as required must be clearly visible or reproduced on the outside of the overpack [7.1.7.1, 7.2.7]	☐	☐	☐
16. The word "Overpack" marked if markings and labels are not visible [7.1.7.1]	☐	☐	☐
17. The total net quantity of carbon dioxide, solid (dry ice) in the overpack [7.1.7.1]	☐	☐	☐
Note: *The Marking and labelling requirements do not apply to ULDs containing dry ice*			
State and Operator Variations			
18. State and operator variations complied with [2.8]	☐	☐	☐

Comments: _____

_____

Checked by: _____

Place: _____ Signature: _____

Date: _____ Time: _____

*** IF ANY BOX IS CHECKED "NO", DO NOT ACCEPT THE SHIPMENT AND GIVE A DUPLICATE COPY OF THIS COMPLETED FORM TO THE SHIPPER.**

CENTERS FOR DISEASE CONTROL AND PREVENTION

DEPARTMENT OF HEALTH AND HUMAN SERVICES

UNITED STATES OF AMERICA

World Health Organization Information on Biosafety

RELEASE TO THE PUBLIC

VERSION 1.0

AUGUST 2014

Interim Infection Prevention and Control Guidance for Care of Patients with Suspected or Confirmed Filovirus Haemorrhagic Fever in Health-Care Settings, with Focus on Ebola

August 2014

CONTENTS

Ref: WHO/HIS/SDS/2014.4

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Key messages for infection prevention and control (IPC) to be applied in health care

- Strengthen and carefully apply standard precautions when providing care to ALL patients regardless of the signs and symptoms they present with.
- Isolate suspected or confirmed hemorrhagic fever (HF) cases in single isolation rooms or cohort them in specific confined areas while rigorously keeping suspected and confirmed cases separate. Assure restricted access to these areas and dedicated equipment.
- Exclusively assign clinical and non-clinical personnel to HF patient care areas.
- Ensure that prior to entering the patient isolation rooms/areas, all visitors and health-care workers rigorously use personal protective equipment (PPE) and perform hand hygiene as indicated in this document. PPE should include at least: gloves, gown, boots/closed shoes with overshoes (and mask and eye protection for splashes).
- Ensure safety of injections and phlebotomy procedures and management of sharps.
- Ensure regular and rigorous environmental cleaning, decontamination of surfaces and equipment, and management of soiled linen and of waste as indicated in this document.
- Ensure safe processing of laboratory samples from suspected or confirmed patients with HF.
- Ensure that the IPC measures indicated in this document are followed while handling dead bodies or human remains of suspected or confirmed patients with HF for post-mortem examination and burial preparation.
- Promptly evaluate, care for, and if necessary, isolate health-care workers or any person exposed to blood or body fluids from suspected or confirmed patients with HF.

INTRODUCTION

This document provides a summary of infection prevention and control (IPC) measures for those providing direct and non-direct care to patients with suspected or confirmed cases of Filovirus haemorrhagic fever (HF), including Ebola or Marburg haemorrhagic fevers, in health-care facilities (HCFs). It also includes some instructions and directions for those managing the implementation of IPC activities. These IPC measures should be applied not only by health-care professionals but by anyone in direct contact with patients (e.g., visitors, family members, volunteers), as well as by those not in contact with patients but potentially exposed the virus through contact with the environment (e.g., c leaners, laundry, house-keepers, security).

This document represents a rapid update of the WHO 2008 *"Interim Infection Control Recommendations for Care of Patients with Suspected or Confirmed Filovirus (Ebola, Marburg) Hemorrhagic Fever"*. This update is based upon review of WHO and other international reference documents being used in the current Ebola outbreak (see references) and international experts' consensus.

Ebola is a severe illness caused by Ebola virus. (http://www.who.int/csr/disease/ebola/en/). It is highly infectious, rapidly fatal, with a death rate of up to 90%, ***but can be prevented.*** It is spread through ***direct contact*** with body fluids like blood, saliva, urine, sperm, etc. of an infected person and by contact with contaminated surfaces or equipment, including linen soiled by body fluids from an infected person. The Ebola virus can be relatively easily eliminated with heat, alcohol-based products, and sodium hypochlorite (bleach) or calcium hypochlorite (bleaching powder) at appropriate concentrations.

If carefully implemented, IPC measures will reduce or stop the spread of the virus and protect health-care workers (HCWs) and others. It is advised that in the affected area(s), a subcommittee for clinical case management is established;[1] as part of this committee, a coordinator(s) should be named to oversee adherence to the IPC measures in each HCF and acts as a focal person to coordinate activities and advise. If available, this person should be the professional in charge of IPC in the HCF.

Case identification and detection, contact tracing and patient clinical assessment and management are not the object of this Guidance document and instructions can be found elsewhere.[1, 2] However, regarding IPC measures to be implemented during interviews for contact tracing and case finding in the community, the following principles should be kept in mind: 1) shaking hands should be avoided; 2) a distance of more than one metre (about 3 feet) should be maintained between interviewer and interviewee; 3) PPE is not required if this distance is assured and when interviewing asymptomatic individuals (e.g., neither fever, nor diarrhoea, bleeding or vomiting) and provided there will be no contact with the environment, potentially contaminated with a possible/probable case; 4) it is advisable to provide workers undertaking contact tracing and case finding in the community with alcohol-based hand rub solutions and instructions to appropriately perform hand hygiene.

1. GENERAL PATIENT CARE

Strengthen and carefully apply ***standard precautions*** [2-4] (Annex 1) when providing care to ALL patients regardless of the signs and symptoms they present with. This is especially important because the initial manifestations of HF may be non-specific. Hand hygiene is the most important measure. Gloves should be worn for any contact with blood or body fluid. Medical mask and goggles or face shield should be used if there is any potential for splashes of blood or body fluids to the face, and cleaning of contaminated surfaces is paramount.

2. DIRECT PATIENT CARE (FOR SUSPECTED OR CONFIRMED PATIENTS WITH HF)

PATIENT PLACEMENT, STAFF ALLOCATION, VISITORS

- Put suspected or confirmed cases in single ***isolation rooms*** with an adjoining dedicated toilet or latrine, showers, sink equipped with running water, soap and single-use towels, alcohol-based hand rub dispensers, stocks of personal protective equipment (PPE), stocks of medicines, good ventilation, screened windows, doors closed and restricted access;[2] if isolation rooms are unavailable, ***cohort*** these patients in specific confined areas while rigorously ***keeping suspected and confirmed cases separate*** and ensure the items listed here for isolation rooms are readily available. Make sure that there is at least 1 meter (3 feet) distance between patient beds.
- Ensure that clinical and non-clinical personnel are assigned exclusively to HF patient care areas and that members of staff do not move freely between the HF isolation areas and other clinical areas during the outbreak.
- Restrict all non-essential staff from HF patient care areas.
- Stopping visitor access to the patient is preferred, but if this is not possible, limit their number to include only those necessary for the patient's well-being and care, such as a child's parent.
- Do not allow other visitors to enter the isolation rooms/areas and ensure that any visitors wishing to observe the patient do so from an adequate distance (approximately 15 m or 50 feet).
- Before allowing visitors to HF patients to enter the HCF, screen them for signs and symptoms of HF.

HAND HYGIENE, PERSONAL PROTECTIVE EQUIPMENT (PPE) AND OTHER PRECAUTIONS

- Ensure that all visitors use PPE and perform hand hygiene as indicated below and are provided with related instructions (Annexes 2, 3, 4) [2, 5, 6] prior to entry into the isolation room/area.
- Ensure that all HCWs (including aides and cleaners) wear PPE (Annexes 2, 3, 4) as appropriate according to the expected level of risk before entering the isolation rooms/areas and having contacts with the patients and/or the environment.
- Personal clothing should not be worn for working in the patient areas. Scrub or medical suits should be worn.

- ***Carefully apply the following precautions*** [3, 7] to avoid any possible unprotected direct contact with blood and body fluids when providing care to any patient with HF, including suspected cases:

  ➔ Perform ***hand hygiene:***
  - before donning gloves and wearing PPE on entry to the isolation room/area,
  - before any clean/aseptic procedures being performed on a patient,
  - after any exposure risk or actual exposure with the patient's blood and body fluids,
  - after touching (even potentially) contaminated surfaces/items/equipment in the patient's surroundings,
  - and after removal of PPE, upon leaving the care area.

  Hand hygiene should be performed within the isolation rooms/areas every time it is needed according to the above indications during care to a patient, along with change of gloves. When caring for patients in the same room, it is essential to organize the complete care to each patient before moving to the next

and to perform hand hygiene between touching the patients. Furthermore, neglecting to perform hand hygiene after removing PPE will reduce or negate any benefits of the protective equipment.
To perform hand hygiene, either use an ***alcohol-based hand rub or soap and running water*** applying the correct technique recommended by WHO (Annex 3).[5] Always perform hand hygiene with soap and running water when hands are visibly soiled. Alcohol-based hand rubs should be made available at every point of care (at the entrance and within the isolation rooms/areas) and are the standard of care. However, if alcohol-based hand rubs are unavailable, perform hand hygiene with soap and running water every time necessary according to the above indications. Alcohol-based hand rubs can be produced locally at the HCF level by following the WHO recommendations and instructions (Annex 5).[8]

- Before entering the isolation rooms/areas, wear ***PPE*** in dedicated changing zone as follows and according to the sequence illustrated in Annex 2:
  - Correctly sized ***gloves*** (non-sterile examination gloves) when entering the patient care area (Annex 3).[6] Consider changing gloves if heavily soiled with blood or any body fluids while providing care to the same patient (perform careful hand hygiene immediately after removal). Always change gloves and perform hand hygiene immediately after removal, when moving from one patient to another while caring for patients in the same room. Consider double gloving when the quality of gloves appears to be poor (e.g., if holes and tears form rapidly during use).
  - A disposable, impermeable ***gown*** to cover clothing and exposed skin.
  - A medical ***mask*** and ***eye protection*** (eye visor or goggles or face shield) to prevent splashes to the nose, mouth and eyes.
  - Closed, puncture and fluid resistant ***shoes*** (e.g. ***rubber boots***) to avoid contamination with blood or other body fluids or accidents with misplaced, contaminated sharp objects. If boots are not available, overshoes should be used but these must be removed while still wearing gloves and with caution to avoid hand contamination (Annex 2).

- When undertaking any strenuous activity (e.g. carrying a patient) or tasks in which contact with blood and body fluids is anticipated (e.g., the patient has symptoms like diarrhoea, bleeding or vomiting and/or the environment could be contaminated with blood or body fluids), in addition to the above-mentioned PPE also use ***double gloving***, and wear a ***waterproof apron*** over the gown if for any reasons your gown is non-impermeable, and disposable overshoes and leg coverings, if boots are not available.
- Avoid aerosol-generating procedures if possible. Wear a ***respirator*** (FFP2 or EN certified equivalent or US NIOSH-certified N95), if any procedures that stimulate coughing or promote the generation of aerosols (e.g., aerosolized or nebulized medication administration, diagnostic sputum induction, bronchoscopy, airway suctioning, endotracheal intubation, positive pressure ventilation via face mask) is planned to be performed.[7]
- Before exiting the isolation room/area, ***carefully remove and dispose of PPE*** (including boots) into waste containers and perform hand hygiene (Annex 2).[2]
- When removing PPE, be careful to avoid any contact between the soiled items (e.g. gloves, gowns) and any area of the face (i.e. eyes, nose or mouth) or non-intact skin.
- ***Do not recycle any single-use disposable PPE.*** However, if the decontamination of goggles and visors is necessary, it is essential that these items should be cleaned with water (± detergent) to remove any organic matter and then immersed fully in 1000 ppm [parts per million] of available chlorine (0.5%) for a minimum of 30 mins (preferably overnight) for decontamination. After decontamination, they should be thoroughly rinsed with water (to remove irritating hypochlorite residues and salt deposits) before re-use. The wipes used for the initial cleaning should be treated as infectious waste; the disinfectant can be safely poured down a sink or drain.[9]
- Carefully ***clean and decontaminate*** reusable equipment (as described below).
- Rigorously use ***dedicated equipment*** (e.g. stethoscopes) for each patient. However, if this is not possible, decontaminate the items between each patient contact. For instance, if the stethoscope has to be used on different patients, it is essential that the full stethoscope (i.e. staff hand contact as well as patient contact surfaces) be thoroughly cleaned first with water and soap using appropriate PPE to remove organic matter and then wiped with alcohol.[9] All waste generated during this decontamination process should be treated as infectious waste (see below).

➔ Items and equipment should not be moved between isolation rooms/areas and other areas of the HCF, unless they are appropriately discarded and disposed. For instance, the patient charts and records should be kept outside the isolation rooms/areas to avoid their contamination.

INJECTION SAFETY AND MANAGEMENT OF SHARPS

- Each patient should have ***exclusively dedicated injection and parenteral medication equipment*** which should be disposed of at the point of care. Syringes, needles or similar equipment should never be reused.
- Limit the use of needles and other sharp objects as much as possible.
- Limit the use of phlebotomy and laboratory testing to the minimum necessary for essential diagnostic evaluation and patient care.[9]
- If the use of sharp objects cannot be avoided, ensure the following precautions are observed:[10]
  - Never replace the cap on a used needle.
  - Never direct the point of a used needle towards any part of the body.
  - Do not remove used needles from disposable syringes by hand, and do not bend, break or otherwise manipulate used needles by hand.
  - Dispose of syringes, needles, scalpel blades and other sharp objects in appropriate, puncture-resistant containers.
- Ensure that puncture-resistant containers for sharps objects are placed as close as possible to the immediate area where the objects are being used ('point of use') to limit the distance between use and disposal, and ensure the containers remain upright at all times. If the sharps container is far, never carry sharps in your hand but place them all in a kidney dish or similar to carry to the sharps container.
- Ensure that the puncture-resistant containers are securely sealed with a lid and replaced when 3/4 full.
- Ensure the containers are placed in an area that is not easily accessible by visitors, particularly children (e.g. containers should not be placed on floors, or on the lower shelves of trolleys in areas where children might gain access).

3. ENVIRONMENTAL CLEANING AND MANAGEMENT OF LINEN

PPE

- ***Wear heavy duty/rubber gloves, impermeable gown and closed shoes (e.g. boots)*** when cleaning the environment and handling infectious waste.
- In addition, wear facial protection (mask and goggle or face shield) and overshoes if boots are unavailable, when undertaking cleaning activities with increased risk of splashes or in which contact with blood and body fluids is anticipated (e.g., cleaning surfaces heavily soiled with vomit or blood or cleaning areas closer than 1 meter/3 feet from a patient with symptoms like diarrhoea, bleeding or vomiting, etc.).

CLEANING PROCESS

- Environmental surfaces or objects contaminated with blood, other body fluids, secretions or excretions should be cleaned and disinfected as soon as possible using standard hospital detergents/disinfectants (e.g. a 0.5% chlorine solution or a solution containing 1 000 ppm available free chlorine)[11]. ***Application of disinfectants should be preceded by cleaning*** to prevent inactivation of disinfectants by organic matter.
- If locally prepared, prepare cleaning and disinfectant solutions every day. Change cleaning solutions and refresh equipment frequently while being used during the day, as they will get contaminated quickly (follow your hospital protocols if available). For preparing chlorine-based solutions, see instructions in Annex 6.
- Clean floors and horizontal work surfaces at least once a day with clean water and detergent. Cleaning with a moistened cloth helps to avoid contaminating the air and other surfaces with air-borne particles. Allow surfaces to dry naturally before using them again.
- Dry sweeping with a broom should never be done. Rags holding dust should not be shaken out and surfaces should not be cleaned with dry rags.
- Cleaning should always be carried out from "clean" areas to "dirty" areas, in order to avoid contaminant transfer.
- ***Do not spray*** (i.e. fog) occupied or unoccupied clinical areas with disinfectant. This is a potentially dangerous practice that has no proven disease control benefit.

MANAGEMENT OF LINEN

- Linen that has been used on patients can be heavily contaminated with body fluids (e.g. blood, vomit) and splashes may result during handling. When handling soiled linen from patients, ***use gloves, gown, closed shoes (e.g., boots) and facial protection (mask and goggle or face shield).***
- Soiled linen should be placed in clearly-labelled, leak-proof bags or buckets at the site of use and the container surfaces should be disinfected (using an effective disinfectant) before removal from the isolation room/area. If there is any solid excrement such as faeces or vomit, scrap off carefully using a flat firm object and flush it down the toilet or in the sluice before linen is placed in its container. If the linen is transported out of the patient room/area for this procedure it should be put in a separate containter – it should never be carried against the body.
- Linen should be then transported directly to the laundry area in its container and laundered promptly with water and detergent.
- For low-temperature laundering, wash linen with detergent and water, rinse and then soak in 0.05% chlorine for approximately 30 minutes. Linen should then be dried according to routine standards and procedures.
- Washing contaminated linen by hand should be discouraged. However, if washing machines are not available or power is not ensured, take the soiled linen out of the container and empty it into a large drum container of hot water and soap. Soak the linen in this drum and make sure it is totally covered with water. Use a stick to stir; then throw out the water and refill the drum with clean water and add bleach 1000ppm and allow to soak for 10–15 minutes. Remove the linen and then rinse in clean water. Remove excess water and spread out to dry. Avoid as much splashing as possible.
- If safe cleaning and disinfection of heavily soiled linen is not possible or reliable, it may be prudent to burn the linen to avoid any unnecessary risks to individuals handling these items.

4. WASTE MANAGEMENT

PPE

- ***Wear heavy duty/rubber gloves, impermeable gown, closed shoes (e.g. boots) and facial protection (mask and goggle or face shield)***, when handling infectious waste (e.g. solid waste or any secretion or excretion with visible blood even if it originated from a normally sterile body cavity). Goggles provide greater protection than visors from splashes that may come from below when pouring liquid waste from a bucket. Avoid splashing when disposing of liquid infectious waste.

WASTE MANAGEMENT PROCEDURES

- Waste should be segregated at point of generation to enable appropriate and safe handling.
- Sharp objects (e.g. needles, syringes, glass articles) and tubing that has been in contact with blood or body fluids should be placed inside puncture resistant waste containers (as described above). These should be located as close as practical to the patient care area where the items are used, similarly in laboratories.
- Collect all solid, non-sharp, infectious waste using leak-proof waste bags and covered bins. Bins should never be carried against the body (e.g. on the shoulder).
- Waste should be placed in a designated pit of appropriate depth (e.g. 2 m or about 7 feet) and filled to a depth of 1–1.5 m (or about 3–5 feet). After each waste load, the waste should be covered with a layer of soil 10 –15 cm deep.
- An incinerator may be used for short periods during an outbreak to destroy solid waste. However, it is essential to ensure that total incineration has taken place. Caution is also required when handling flammable material and when wearing gloves due to the risk of burn injuries if gloves are ignited.
- Placenta and anatomical samples should be buried in a separate pit.
- The area designated for the final treatment and disposal of waste should have controlled access to prevent entry by animals, untrained personnel or children.
- Waste, such as faeces, urine and vomit, and liquid waste from washing, can be disposed of in the sanitary sewer or pit latrine. No further treatment is necessary.

Table. Summary table for implementation of IPC best practices during direct patient care and related activities

What?	How?	Who is responsible?
Create isolation rooms or areas.	- Identify single rooms and prioritise these for patients with known or suspected Ebola virus. - Refer to guidance on setting up an isolation area.[2]	- Coordinator or IPC staff to identify areas/rooms for patient placement. - Health workers to adhere to recommendations and report to the coordinator when a patient is not placed in an isolation room/area.
Restrict all non-essential staff from HF patient care rooms/areas.	- Ensure that clinical and non-clinical personnel are assigned exclusively to patient care areas and that members of staff do not move freely between these areas and other clinical areas during the outbreak. - Cohort staff between areas with suspected and those with confirmed HF patients. - Use signage to alert restrictions of staff. - Maintain a log of persons entering the room.	- Coordinator and/or IPC staff.
Limit the number of visitors allowed access to the patient.	- Use signage and other communications to alert restrictions of visitors. Make simple messages understandable for the public but also be careful to avoid stigmatization. - Maintain a log of persons entering the room.	- Coordinator and/or IPC staff - Involve patient or community representatives, if available. - Health workers to adhere to recommendations and report to the coordinator when they are not followed.
Ensure that all staff and visitors correctly use and remove recommended personal protective equipment (PPE).	- Ensure the equipment is always available and promptly at the isolation rooms/areas entry. - Provide staff and visitors with instructions on the use and correct removal of PPE through training and reminder posters.	- Coordinator and/or IPC staff - Involve patient or community representatives, if available. - Health workers to adhere to recommendations and report to the coordinator when they are not followed. - Another staff member should be assigned to supervise the sequence of putting on and removing PPE by his/her colleague.

What?	How?	Who is responsible?
Ensure that all staff and visitors perform hand hygiene according to the above recommendations. These hand hygiene actions should be performed when recommended even if PPE is worn.	- Provide staff and visitors with instructions on the importance of hand hygiene best practices through training and reminder posters. - Ensure continuous availability of alcohol-based handrub and soap, water and single-use towels at the isolation room/areas entry and at the point of care.	- Coordinator and/or IPC staff. - Involve patient or community representatives, if available. - Health workers to adhere to recommendations and report to the coordinator when they are not followed.
Limit the use of needles and other sharp objects as much as possible. If this cannot be avoided see instructions in the text.	- Provide staff and carers with instructions on the essential use of needles and sharps through training and reminder posters. - Ensure the equipment is available to do this.	- Health workers to adhere to recommendations.
Dispose of needles and other sharp objects safely.	- Provide staff and carers with instructions on the safe disposal of sharps through training and reminder posters. - Ensure the equipment is available to do this.	- Health workers to adhere to recommendations and report to the coordinator when they are not followed.
Create system of safe management of waste and linen.	- Provide staff and visitors/carers with instructions on the safe management and disposal of waste and linen through training and reminder posters. - Ensure the equipment is available to do this.	- Health workers to adhere to recommendations and report to the coordinator when they are not followed.
Limit the use of phlebotomy and laboratory testing to the minimum necessary for essential diagnostic evaluation and patient care.	- Provide staff with training and visual instructions on the need for essential phlebotomy and lab testing.	- Health workers to adhere to recommendations.
Only take a patient out of their room/care area if they are free of virus, or for essential, life-saving tests.	- Provide staff with training and visual instructions on the appropriate times to take the patient from their care area and on precautions to take.	- Health workers to adhere to recommendations and report to the coordinator when they are not followed.
Undertake cleaning of the environment and patient care equipment safely following recommendations in the text.	- Provide staff and visitors/carers with instructions on cleaning through training and reminder posters. - Ensure the equipment is available to undertake recommended cleaning.	- Health workers to adhere to recommendations and report to the coordinator when they are not followed.

IPC = infection prevention and control; PPE = personal protective equipment

5. NON-PATIENT CARE ACTIVITIES (FOR SUSPECTED OR CONFIRMED PATIENTS WITH HF)

A. DIAGNOSTIC LABORATORY ACTIVITIES

- For procedures to safely collect blood or other samples from persons suspected or confirmed to be infected, follow the instructions provided by WHO.[9]
- All laboratory sample processing must take place under a safety cabinet or at least a fume cabinet with exhaust ventilation. Do not carry out any procedure on the open bench.
- Activities such as micro-pipetting and centrifugation can mechanically generate fine aerosols that might pose a risk of transmission of infection through inhalation as well as the risk of direct exposure.
- Laboratory personnel handling potential HF clinical specimens should wear closed shoes with overshoes or boots, gloves, a disposable, impermeable gown, eye protection or face shields, and particulate respirators (e.g., FFP2, or EN certified equivalent, or US NIOSH-certified N95) , or powered air purifying respirators (PAPR) when aliquotting, performing centrifugation or undertaking any other procedure that may generate aerosols.
- When removing PPE, avoid any contact between the soiled items (e.g. gloves, gowns) and any area of the face (i.e. eyes, nose or mouth).
- Do not hang up the apron or gown for reuse- discard immediately.
- Perform hand hygiene immediately after the removal of PPE used during specimen handling and after any contact with potentially contaminated surfaces even when PPE is worn.
- Place specimens in clearly-labelled, non-glass, leak-proof containers and deliver directly to designated specimen handling areas.
- Disinfect all external surfaces of specimen containers thoroughly (using an effective disinfectant) prior to transport.

B. MOVEMENT AND BURIAL OF HUMAN REMAINS

- The coordinator and/or the infection prevention and control staff should be consulted for any decision making on movement and burial of human remains.
- For this topic, see also the WHO "Interim manual-Ebola and Marburg virus disease epidemics: preparedness, alert, control, and evaluation".[1]
- The handling of human remains should be kept to a minimum. The following recommendations should be adhered to in principle, but may need some adaptation to take account of cultural and religious concerns:
  - ***Wear PPE*** (impermeable gown, mask, eye protection and double gloves) ***and closed shoes or boots*** to handle the dead body of a suspected or confirmed case of HF. Plug the natural orifices. Place the body in a double bag, wipe over the surface of each body bag with a suitable disinfectant (e.g., 0.5% chlorine solution) and seal and label with the indication of highly-infectious material. Immediately move the body to the mortuary.
  - PPE should be put on at the site of collection of human remains, worn during the process of collection and placement in body bags, and should be removed immediately after. Hand hygiene should be performed immediately following the removal of PPE.
  - Remains should not be sprayed, washed or embalmed. Any practice of washing the remains in preparation of "clean burials" should be discouraged.
  - Only trained personnel should handle remains during the outbreak.
  - PPE is not required for individuals driving or riding a vehicle to collect human remains, provided that drivers or riders will not be handling a dead body of a suspected or confirmed case of HF.
  - After wrapping in sealed, leak-proof material, remains should be placed inside a coffin if possible, and buried promptly.

C. POST-MORTEM EXAMINATIONS

- The coordinator and/or the IPC staff should be consulted for any decision making on post-mortem examinations.
- Post-mortem examination of HF patient remains should be limited to essential evaluations only and should be performed by trained personnel.

- Personnel examining remains should ***wear eye protection, mask, double gloves, disposable, impermeable gowns, and closed shoes or boots.***
- In addition, personnel performing autopsies of known or suspected HF patients should wear a particulate respirator (e.g., FFP2, or EN certified equivalent, or US NIOSH-certified N95) or a PAPR.
- When removing PPE, avoid any contact between soiled gloves or equipment and the face (i.e. eyes, nose or mouth).
- Hand hygiene should be performed immediately following the removal of PPE.
- Place specimens in clearly-labelled, non-glass, leak-proof containers and deliver directly to designated specimen handling areas.
- All external surfaces of specimen containers should be thoroughly disinfected (using an effective disinfectant) prior to transport.
- Tissue or body fluids for disposal should be carefully placed in clearly marked, sealed containers for incineration.

D. MANAGING EXPOSURE TO VIRUS THROUGH BODY FLUIDS INCLUDING BLOOD

- Persons including HCWs with percutaneous or muco-cutaneous exposure to blood, body fluids, secretions, or excretions from a patient with suspected or confirmed HF should ***immediately and safely*** *stop any current tasks, leave the patient care area, and safely remove PPE*. Remove PPE carefully according to the steps indicated in this document (Annex 2) because exposure during PPE removal can be just as dangerous for nosocomial transmission of HF. Immediately after leaving the patient care area, ***wash*** the affected skin surfaces or the percutaneous injury site with soap and water . Accordingly, irrigate mucous membranes (e.g. conjunctiva) with copious amounts of water or an eyewash solution, and not with chlorine solutions or other disinfectants.
- Immediately report the incident to the local coordinator. This is a time-sensitive task and should be performed as soon as the HCW leaves the patient care unit.
- Exposed persons should be ***medically evaluated*** including for other potential exposures (e.g., HIV, HCV) and ***receive follow-up care***, including fever monitoring, twice daily for 21 days after the incident. Immediate consultation with an expert in infectious diseases is recommended for any exposed person who develops fever within 21 days of exposure.
- HCWs suspected of being infected should be cared for/isolated, and the same recommendations outlined in this document must be applied until a negative diagnosis is confirmed.
- Contact tracing and follow-up of family, friends, co-workers and other patients, who may have been exposed to Ebola virus through close contact with the infected HCW is essential.

REFERENCES

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2 Clinical Management of Patients with Viral Haemorrhagic Fever: A pocket Guide for the Front-line Health Worker. World Health Organization, Geneva, 2014.

3 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. Centers for Disease Control and Prevention, Atlanta, GA, 2007; Available from: http://www.cdc.gov/HAI/prevent/prevent_pubs.html

4 Standard precautions in health care AIDE-MEMOIRE. World Health Organization, Geneva, 2007; Available from: http://www.who.int/csr/resources/publications/standardprecautions/en/.

5 Hand Hygiene Posters. World Health Organization, Geneva, 2009. ; Available from: http://www.who.int/gpsc/5may/tools/workplace_reminders/en/

6 Glove Use Information Leaflet. World Health Organization, Geneva, 2009.; Available from: http://www.who.int/gpsc/5may/tools/training_education/en/

7 Infection Prevention and Control Recommendations for Hospitalized Patients with Known or Suspected Ebola Hemorrhagic Fever in U.S. Hospitals. Centers for Disease Control and Prevention, Atlanta, GA; Available from: http://www.cdc.gov/vhf/ebola/hcp/infection-prevention-and-control-recommendations.html

8 Guide to Local Production: WHO-recommended Handrub Formulations. World Health Organization, Geneva, 2010; Available from: http://www.who.int/gpsc/5may/tools/system_change/en/.

9 Hoffman PN, Bradley C, Ayliffe GAJ, Health Protection Agency (Great Britain). Disinfection in healthcare. 3rd ed. Malden, Mass: Blackwell Pub.; 2004.

10 How to safely collect blood samples from persons suspected to be infected with highly infectious blood-borne pathogens (e.g. Ebola) World Health Organization.

11 WHO best practices for injections and related procedures toolkit. World Health Organization, Geneva, 2010; Available from: http://www.who.int/injection_safety/toolbox/9789241599252/en/

12 Management of Hazard Group 4 viral haemorrhagic fevers and similar human infectious diseases of high consequence. Department of Health, United Kingdom, 2012; Available from: http://www.dh.gov.uk/publications.

Annex 1. Standard Precautions in health Care – AIDE MEMOIRE
KEY ELEMENTS AT A GLANCE

1. Hand hygiene[1]

How to perform hand hygiene:

- Clean your hands by **rubbing them with an alcohol-based formulation**, as the preferred mean for routine hygienic hand antisepsis if hands are not visibly soiled. It is faster, more effective, and better tolerated by your hands than washing with soap and water.
- **Wash your hands with soap and water** when hands are visibly dirty or visibly soiled with blood or other body fluids or after using the toilet.
- If exposure to potential spore-forming pathogens is strongly suspected or proven, including outbreaks of *Clostridium difficile*, hand washing with soap and water is the preferred means.

Summary technique:[1]

- Hand washing (40–60 sec): wet hands and apply soap; rub all surfaces; rinse hands and dry thoroughly with a single use towel; use towel to turn off faucet.
- Hand rubbing (20–30 sec): apply enough product to cover all areas of the hands; rub all surfaces until dry.

Summary indications:[1]

1. Before touching a patient: Clean your hands before touching a patient when approaching him/her*

2. Before clean / aseptic procedure: Clean your hands immediately before accessing a critical site with infectious risk for the patient (e.g. a mucous membrane, non-intact skin, an invasive medical device)*

3. After body fluid exposure risk: Clean your hands as soon as the task involving an exposure risk to body fluids has ended (and after glove removal)*

4. After touching a patient: Clean your hands when leaving the patient's side after having touched the patient*

5. After touching patient surroundings: Clean your hands after touching any object or furniture when living the patient surroundings, without having touched the patient*

2. Gloves

- Wear GLOVES when touching blood, body fluids, secretions, excretions, mucous membranes, nonintact skin.
- Change GLOVES between tasks and procedures on the same patient after contact with potentially infectious material.
- Remove THEM after use, before touching non-contaminated items and surfaces, and before going to another patient. Perform hand hygiene immediately after removal.

3. Facial protection (eyes, nose, and mouth)

- Wear (1) a surgical or procedure mask and eye protection (eye visor, goggles) or (2) a face shield to protect mucous membranes of the eyes, nose, and mouth during activities that are likely to generate splashes or sprays of blood, body fluids, secretions, and excretions.

4. Gown

- Wear to protect skin and prevent soiling of clothing during activities that are likely to generate splashes or sprays of blood, body fluids, secretions, or excretions.
- Remove soiled gown as soon as possible, and per-form hand hygiene.

5. Prevention of needle stick and injuries from other sharp instruments[2]

Use care when:

- Handling needles, scalpels, and other sharp instruments or devices.

6. Respiratory hygiene and cough etiquette

Persons with respiratory symptoms should apply source control measures:

- Cover their nose and mouth when coughing/sneezing with tissue or mask, dispose of used tissues and masks, and perform hand hygiene after contact with respiratory secretions.

Health-care facilities should:

- Place acute febrile respiratory symptomatic patients at least 1 metre (3 feet) away from others in common waiting areas, if possible.
- Post visual alerts at the entrance to health-care facilities instructing persons with respiratory symptoms to practise respiratory hygiene/cough etiquette.
- Consider making hand hygiene resources, tissues and masks available in common areas and areas used for the evaluation of patients with respiratory illnesses.

7. Environmental cleaning

- Use adequate procedures for the routine cleaning and disinfection of environmental and other frequently touched surfaces.

8. Linens

Handle, transport, and process used linen in a manner which:

- Prevents skin and mucous membrane exposures and contamination of clothing.
- Avoids transfer of pathogens to other patients and or the environment.

9. Waste disposal

- Ensure safe waste management.
- Treat waste contaminated with blood, body fluids, secretions and excretions as clinical waste, in accordance with local regulations.
- Human tissues and laboratory waste that is directly associated with specimen processing should also be treated as clinical waste.
- Discard single use items properly.

10. Patient care equipment

- Handle equipment soiled with blood, body fluids, secretions, and excretions in a manner that prevents skin and mucous membrane exposures, contamination of clothing, and transfer of pathogens to other patients or the environment.
- Clean, disinfect, and reprocess reusable equipment appropriately before use with another patient.
- Cleaning used instruments.
- Disposing of used needles and other sharp instruments.

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1 For more details, see:1) WHO Guidelines on Hand Hygiene in Health Care, 2009, available at: http://www.who.int/gpsc/5may/tools/en/. 2) "Hand Hygiene: Why, How & When?", available at http://www.who.int/gpsc/5may/tools/training_education/en/

2 The SIGN Alliance at: http://www.who.int/injection_safety/sign/en/

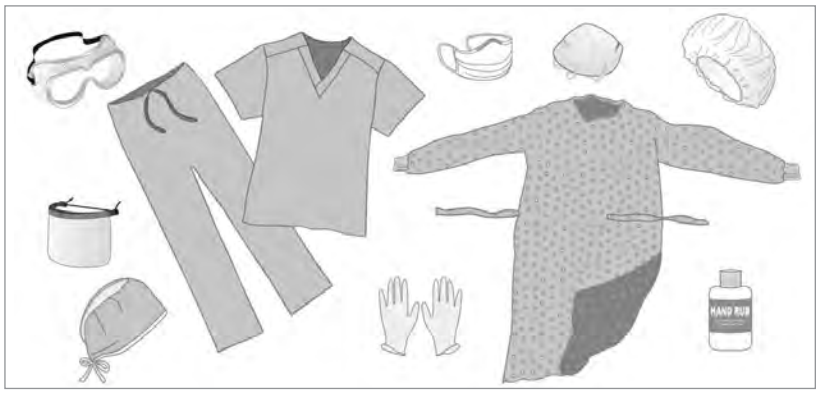
***NOTE:** Hand hygiene must be performed in all indications described regardless of whether gloves are used or not.

Annex 2.
Steps to put on Personal Protective Equipment (PPE)

1 Always put on essential required PPE when handling either a suspect, probable or confirmed case of VHF. Gather all the necessary items of the PPE beforehand.

2 The dressing and undressing of PPE should be supervised by another trained member of the team. These instructions should be displayed on the wall in the dressing and undressing room. Steps to put on essential required PPE.

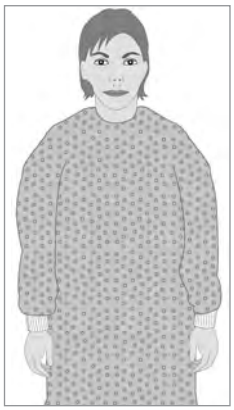
3 Put on the scrub suit in the changing room.



4 Put on gum boots; If not available, make sure you have closed, puncture and fluid resistant shoes and put on overshoes.

OR, IF BOOTS UNAVAILABLE

5 Place the gown over the scrubs.



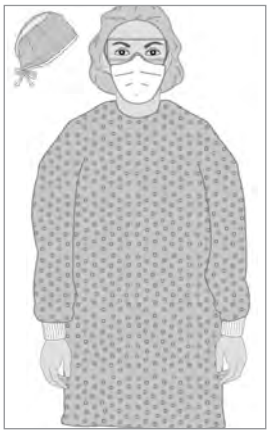
6 Put on face protection:

6a Put on a medical mask.

6b Put on goggles or a face shield

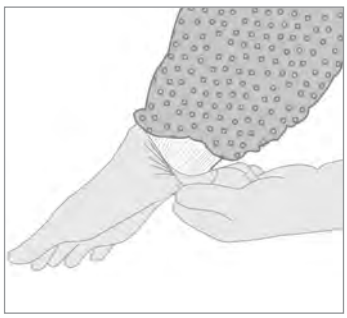
OR

7 If you have any abrasions on your scalp or you have concern for splashing fluids, also place a head cover at this time.



8 Perform hand hygiene.

9 Put on gloves* (over cuff).



10 If a impermeable gown is not available and you expect to undertake any strenuous activity (e.g. carrying a patient) or tasks in which contact with blood and body fluids, place waterproof apron over gown.

Whilst wearing PPE:

- Avoid touching or adjusting PPE
- Remove gloves if they become torn or damaged
- Change gloves between patients
- Perform hand hygiene before donning new gloves

* Use ***double gloves*** if any strenuous activity (e.g. carrying a patient or handling a dead body) or tasks in which contact with blood and body fluids are anticipated. Use ***heavy duty/rubber gloves*** for environmental cleaning and waste management.

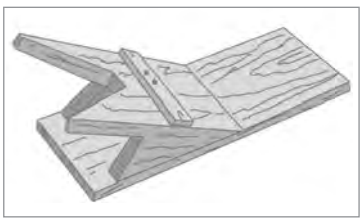
Steps to remove PPE

1 Peel off plastic apron and dispose of safely, (if the apron is to be reused, place in a container with disinfectant)

2 If wearing protective overshoes, please remove them with your gloves still on. (If wearing gum boots, see step 4).

3 Remove gown and gloves and roll inside-out and dispose of safely.

4 If wearing rubber boots, remove them (ideally using the boot remover) without touching them with your hands. Place the removed boots into a container with disinfectant.



5 Perform hand hygiene.

6 If wearing a head covering, remove it now (from behind head).

7 Remove face protection:
7a Remove face shield or goggles (from behind head). Place eye protection in a separate container for reprocessing. **OR**

7b Remove mask from behind head.

8 Perform hand hygiene.

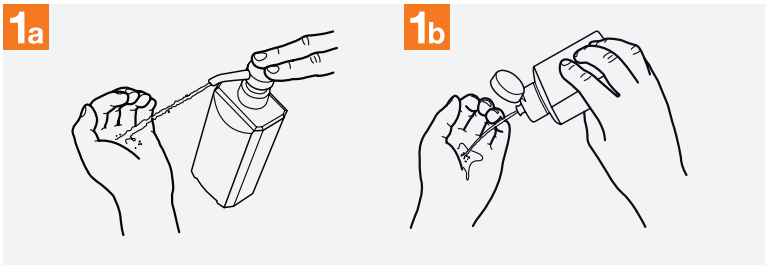
Source: Modified from Clinical Management of Patients with Viral Haemorrhagic Fever: A pocket Guide for the Front-line Health Worker. World Health Organization, 2014

How to Handrub?

RUB HANDS FOR HAND HYGIENE! WASH HANDS WHEN VISIBLY SOILED

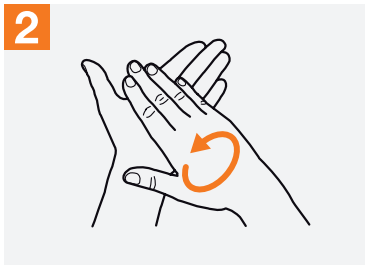
Duration of the entire procedure: 20-30 seconds

1a 1b



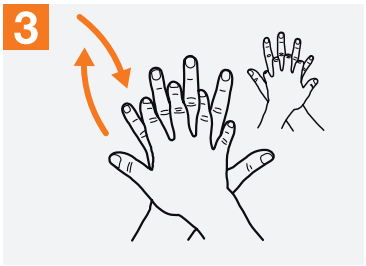
Apply a palmful of the product in a cupped hand, covering all surfaces;

2



Rub hands palm to palm;

3



Right palm over left dorsum with interlaced fingers and vice versa;

4

Palm to palm with fingers interlaced;

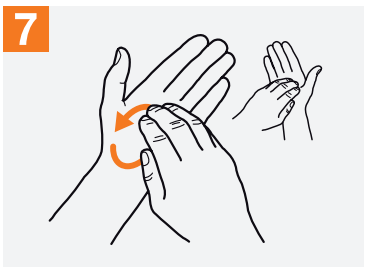
5

Backs of fingers to opposing palms with fingers interlocked;

6

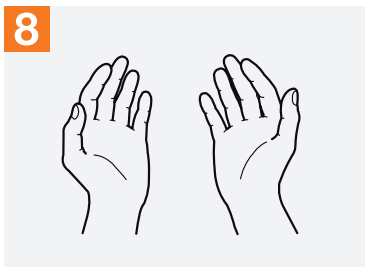
Rotational rubbing of left thumb clasped in right palm and vice versa;

7



Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;

8



Once dry, your hands are safe.

How to Handwash?

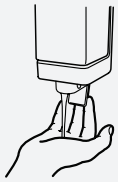
WASH HANDS WHEN VISIBLY SOILED! OTHERWISE, USE HANDRUB

Duration of the entire procedure: 40-60 seconds

0

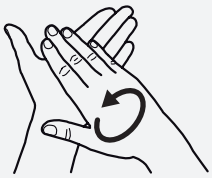
Wet hands with water;

1



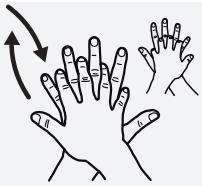
Apply enough soap to cover all hand surfaces;

2



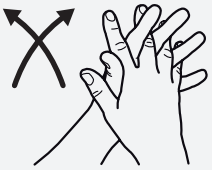
Rub hands palm to palm;

3



Right palm over left dorsum with interlaced fingers and vice versa;

4

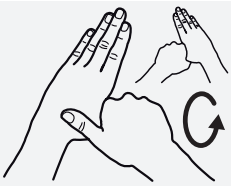


Palm to palm with fingers interlaced;

5

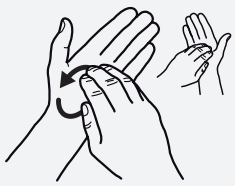
Backs of fingers to opposing palms with fingers interlocked;

6



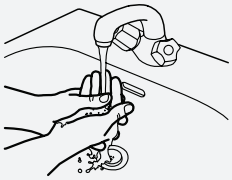
Rotational rubbing of left thumb clasped in right palm and vice versa;

7



Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;

8

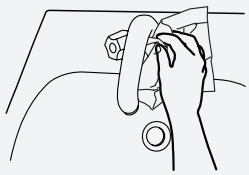


Rinse hands with water;

9

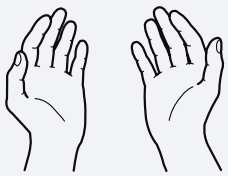
Dry hands thoroughly with a single use towel;

10



Use towel to turn off faucet;

11



Your hands are now safe.

Source: Hand Hygiene Posters. World Health Organization, Geneva, 2009; Available from: http://www.who.int/gpsc/5may/tools/workplace_reminders/en/

Annex 4.
Technique for donning and removing non-sterile examination gloves

When the hand hygiene indication occurs before a contact requiring glove use, perform hand hygiene by rubbing with an alcohol-based handrub or by washing with soap and water.

I. HOW TO DON GLOVES:

1. Take out a glove from its original box

2. Touch only a restricted surface of the glove corresponding to the wrist (at the top edge of the cuff)

3. Don the first glove

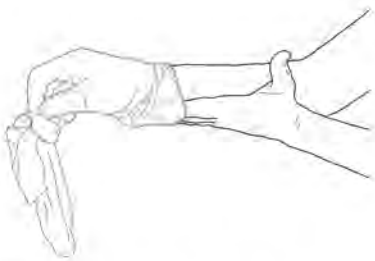
4. Take the second glove with the bare hand and touch only a restricted surface of glove corresponding to the wrist

5. To avoid touching the skin of the forearm with the gloved hand, turn the external surface of the glove to be donned on the folded fingers of the gloved hand, thus permitting to glove the second hand

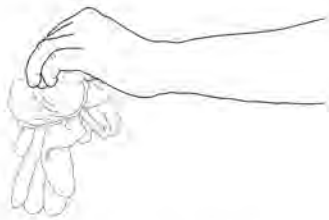
6. Once gloved, hands should not touch anything else that is not defined by indications and conditions for glove use

II. HOW TO REMOVE GLOVES:

1. Pinch one glove at the wrist level to remove it, without touching the skin of the forearm, and peel away from the hand, thus allowing the glove to turn inside out



2. Hold the removed glove in the gloved hand and slide the fingers of the ungloved hand inside between the glove and the wrist. Remove the second glove by rolling it down the hand and fold into the first glove



3. Discard the removed gloves

4. Then, perform hand hygiene by rubbing with an alcohol-based handrub or by washing with soap and water

Source: Glove Use Information Leaflet. World Health Organization, Geneva, 2009. Available from: http://www.who.int/gpsc/5may/tools/training_educational/en/

GUIDE TO LOCAL PRODUCTION

This is intended to guide a local producer in the actual preparation of the formulation.

Materials required (small volume production)

REAGENTS FOR FORMULATION 1:

- Ethanol 96%
- Hydrogen peroxide 3%
- Glycerol 98%
- Sterile distilled or boiled cold water

REAGENTS FOR FORMULATION 2:

- Isopropyl alcohol 99.8%
- Hydrogen peroxide 3%
- Glycerol 98%
- Sterile distilled or boiled cold water

- 10-litre glass or plastic bottles with screw-threaded stoppers (1), or
- 50-litre plastic tanks (preferably in polypropylene or high density polyethylene, translucent so as to see the liquid level) (2), or
- Stainless steel tanks with a capacity of 80–100 litres (for mixing without overflowing) (3, 4)
- Wooden, plastic or metal paddles for mixing (5)
- Measuring cylinders and measuring jugs (6)
- Plastic or metal funnel
- 100 ml and 500 ml plastic bottles with leak-proof tops (7)
- An alcoholometer: the temperature scale is at the bottom and the ethanol concentration (percentage v/v and w/w) at the top (8)

NOTE

- Glycerol: used as humectant, but other emollients may be used for skin care, provided that they are cheap, widely available and miscible in water and alcohol and do not add to toxicity, or promote allergy.
- Hydrogen peroxide: used to inactivate contaminating bacterial spores in the solution and is not an active substance for hand antisepsis.
- Any further additive to both formulations should be clearly labelled and be non-toxic in case of accidental ingestion.
- A colorant may be added to allow differentiation from other fluids, but should not add to toxicity, promote allergy, or interfere with antimicrobial properties. The addition of perfumes or dyes is not recommended due to risk of allergic reactions.

General information

Labelling should be in accordance with national guidelines and should include the following:

- Name of institution, date of production and batch number
- WHO-recommended handrub solution
- For external use only
- Avoid contact with eyes
- Keep out of the reach of children
- Use: Apply a palmful of alcohol-based handrub and cover all surfaces of the hands. Rub hands until dry.
- Composition: ethanol or isopropanol, glycerol and hydrogen peroxide
- Flammable: keep away from flame and heat


1

2

3


4


5


6


7


8

Production and storage facilities:

- Production and storage facilities should ideally be air conditioned or cool rooms. ***No naked flames or smoking should be permitted in these areas***.
- WHO-recommended handrub formulations should not be produced in quantities exceeding 50-litres locally or in central pharmacies lacking specialised air conditioning and ventilation.
- Since undiluted ethanol is highly flammable and may ignite at temperatures as low as 10°C, production facilities should directly dilute it to the above-mentioned concentration. The flashpoints of ethanol 80% (v/v) and of isopropyl alcohol 75% (v/v) are 17.5°C and 19°C, respectively.
- National safety guidelines and local legal requirements must be adhered to the storage of ingredients and the final product.

METHOD: 10-LITRE PREPARATIONS

These can be prepared in 10-litre glass or plastic bottles with screw-threaded stoppers.

Recommended amounts of products:

FORMULATION 1:

- Ethanol 96%: **8333 ml**
- Hydrogen peroxide 3%: **417 ml**
- Glycerol 98%: **145 ml**

FORMULATION 2:

- Isopropyl alcohol 99.8%: **7515 ml**
- Hydrogen peroxide 3%: **417 ml**
- Glycerol 98%: **145 ml**

Final products:

FORMULATION 1:
Final concentrations:

- Ethanol 80% (v/v)
- Glycerol 1.45% (v/v)
- Hydrogen peroxide 0.125% (v/v)

FORMULATION 2:
Final concentrations:

- Isopropyl alcohol 75% (v/v)
- Glycerol 1.45% (v/v)
- Hydrogen peroxide 0.125% (v/v)

Step by step preparation:

1. The alcohol for the formula to be used is poured into the large bottle or tank up to the graduated mark.

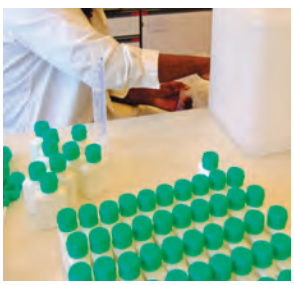
2. Hydrogen peroxide is added using the measuring cylinder.

3. Glycerol is added using a measuring cylinder. As glycerol is very viscous and sticks to the wall of the measuring cylinder, it should be rinsed with some sterile distilled or cold boiled water and then emptied into the bottle/tank.

4. The bottle/tank is then topped up to the 10-litre mark with sterile distilled or cold boiled water.

5. The lid or the screw cap is placed on the tank/bottle as soon as possible after preparation, in order to prevent evaporation.

6. The solution is mixed by shaking gently where appropriate or by using a paddle.

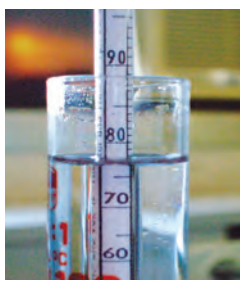


7. Immediately divide up the solution into its final containers (e.g. 500 or 100 ml plastic bottles), and place the bottles in quarantine for 72 hours before use. This allows time for any spores present in the alcohol or the new/re-used bottles to be destroyed.

Quality control

1. Pre-production analysis should be made every time an analysis certificate is not available to guarantee the titration of alcohol (i.e. local production). Verify the alcohol concentration with the alcoholmeter and make the necessary adjustments in volume in the preparation formulation to obtain the final recommended concentration.

2. Post-production analysis is mandatory if either ethanol or an isopropanol solution is used. Use the alcoholmeter to control the alcohol concentration of the final use solution. The accepted limits should be fixed to ± 5% of the target concentration (75%–85% for ethanol).



3. The alcoholmeter shown in this information pamphlet is for use with ethanol; if used to control an isopropanol solution, a 75% solution will show 77% (± 1%) on the scale at 25°C.

Source: Guide to Local Production: WHO-recommended Handrub Formulations, http://www.who.int/gpsc/5may/tools/system_change/en/

Annex 6.
How to make chlorine solutions for environmental disintection

Example I - Using Liquid Bleach

Chlorine in liquid bleach comes in different concentrations. Any concentration can be used to make a dilute chlorine solution by applying the following formula:

$$\left[\frac{\%\text{ chlorine in liquid bleach}}{\%\text{ chlorine desired}}\right] - 1 = \text{Total parts of water for each part bleach}\dagger$$

Example: To make a 0.5% chlorine solution from 3.5%‡ bleach:

$$\left[\frac{3.5\%}{0.5\%}\right] - 1 = 7 - 1 = 6 \text{ parts water for each part bleach}$$

Therefore, you must add 1 part 3.5% bleach to 6 parts water to make a 0.5% chlorine solution.

† "Parts" can be used for any unit of measure (e.g. ounce, litre or gallon) or any container used for measuring, such as a pitcher.

‡ In countries where French products are available, the amount of active chlorine is usually expressed in degrees chlorum. One degree chlorum is equivalent to 0.3% active chlorine.

Example II - Using Bleach Powder

If using bleach powder, † calculate the amount of bleach to be mixed with each litre of water by using the following formula:

$$\left[\frac{\%\text{ chlorine desired}}{\%\text{ chlorine in bleach powder}}\right] \times 1\,000 = \text{Grams of bleach powder for each litre of water}$$

Example: To make a 0.5% chlorine solution from calcium hypochlorite (bleach) powder containing 35% active chlorine:

$$\left[\frac{0.5\%}{35\%}\right] \times 1\,000 = 0.0143 \times 1\,000 = 14.3$$

Therefore, you must dissolve 14.3 grams of calcium hypochlorite (bleach) powder in each litre of water used to make a 0.5% chlorine solution.

† When bleach powder is used; the resulting chlorine solution is likely to be cloudy (milky).

Example III - Formula for Making a Dilute Solution from a Concentrated Solution

$$\text{Total Parts (TP) } (H_2O) = \left[\frac{\%\text{ Concentrate}}{\%\text{ Dilute}}\right] - 1$$

Example: To make a dilute solution (0.1%) from 5% concentrated solution.

$$\text{Calculate TP } (H_2O) = \left[\frac{5.0\%}{0.1\%}\right] - 1 = 50 - 1 = 49$$

Take 1 part concentrated solution and add to 49 parts boiled (filtered if necessary) water.

Source:

AVSC International (1999). Infection Prevention Curriculum. Teacher's Manual. New York, p.267.